

British university upheaval (contd)

The Swinnerton-Dyer questionnaire to British universities should be regarded not as a threat but an opportunity. For the first time in years, institutions have an incentive to be different from each other.

ONLY one thing is certain about the questionnaire put out to all British universities by Sir Peter Swinnerton-Dyer, the new chairman of the University Grants Committee: it will generate a huge flood of correspondence before the closing date at the end of March. Universities as such are required to answer only two of the 29 questions that have been put to them, dealing respectively with plans for change within the confines of present budgets and if university budgets were slowly to shrink while student numbers remained constant. Many universities will doubtless regard these questions as dangerous traps. To outline adventurous plans for development within present constraints will seem like an admission that present restraints are not, after all, that cramping, while even to admit (in reply to the second question) that survival might be compatible with a shrinking budget would be to invite just such a calamity. This is why prudent universities will begin by questioning the assumptions on which the two questions have been based, and which are helpfully spelled out in the letter from the grants committee.

On the face of things, the difficulties of the past few years are likely to be outdone by what lies ahead. For demographic reasons, the population of 18-year-olds in Britain is certain to fall between the closing years of this decade and the end of the century. There can be no quibbling about that gloomy prospect, for the people concerned have already been born. There is more room for argument about the assumptions that the percentage of young people entering universities (and higher education as whole) will remain substantially constant and that there will be no significant shift in the pattern of higher education towards undergraduate courses occupying four years, not just three. Indeed, the first assumption can properly be challenged on social grounds, the second by the simple observation that if the British educational system is ever to produce less narrow specialists, it will find that three years are not enough for teaching most undergraduates what they are at present expected to know on graduation. These assertions will be written off as platitudes, and there will be many who will say that impoverished Britain cannot possibly afford such extravagances. But can a nation bent on rebuilding virtually the whole of its industrial base afford not to afford them?

Complaints

The more particular complaints that individual universities must make against the assumptions on which the two questions are asked of them are dangerous in a different sense, for they will imply a markedly different relationship between individual universities and the central government on the one hand and other universities on the other. In the old days, before the budget cuts of the past three spartan years were decreed, there was a genteel understanding that the scale and character of a university's operations were determined by various signals emanating from the grants committee, among which the annual recurrent budget was the chief. Recognizing its obligation towards research (and that its own prosperity would be determined by its academics' success in this respect), a university would do its best to see that laboratories were reasonably well equipped, and at the same time would not compete over-zealously for students for fear of burdening its teachers with too much teaching.

Now all that has changed. The past few years have shown that universities have been skimping on research to such a degree that

grant-making bodies have sometimes to supplement research grants with what are essentially overhead payments for the provision of basic equipment. And competition for students (the numbers game) has been outlawed by the decision three years ago by the grants committee that each university should live with a predetermined quota of students. The second restriction is especially iniquitous. Universities should tell the grants committee that they must be freed from this restriction (and from the diseconomies that it brings). Indeed, the ideal is that they should be given an incentive to compete for students, perhaps by a reversal of last year's decision that the notional payment of university fees by local authorities should be chopped in half. And they should then be reminded that competition means that there must be losers as well as winners. Will they have the courage to invite such circumstances upon themselves? And what if such prizes as there are in the years ahead go predominantly only to the universities that show a little nerve?

Courage

The case for courage is even broader. For years it has been plain that diversity, and mechanisms that might encourage it, is the most urgent need within the British university system. In the past, the influence of the grants committee has been in the direction of uniformity, sometimes by the explicit application of what might be called Oxbridge standards to the affairs of other universities, more often by its mere existence. The one potential benefit that might be wrung from the continuing crisis in British higher education is the opportunity it provides for breaking out of the pattern that has become traditional. This is a time when universities that consider they have a special role to play in some field of education or scholarship, or which consider that they would prefer to teach four-year courses, perhaps on the Scottish pattern, or to educate more students than at present — and trust to their own wits to raise research support elsewhere — should say so clearly. With a little luck, they can hope to be heard against the hubbub there will be in the next few months. And there is little chance that they will be penalized for seeming different.

This line of argument bears on the most contentious of the other questions raised by the grants committee — whether its support for university research should be earmarked as such. On the face of things, this would be a means of making sure that the universities' capacity for carrying out research is not steadily eroded by neglectful university administrations, postponing hard decisions about the survival of academic departments (or even academics) by the use of money that should go for research support. While the Advisory Board for the Research Councils has come out against this stratagem, many academics are in favour, often on the grounds that the universities to which they personally belong would probably benefit by the discrimination that it is assumed would follow. But that argument is partly an illusion. The calculation of how much of the general grants committee budget is intended for research support includes a notional allowance for the part of academic staff salaries supposed to be spent on research, amounts which are certain to fluctuate as widely in the coming years as in the past three. Moreover, earmarking would prevent universities that have something new to offer the general pattern of British higher education from making a case to be allowed to do so. On balance, regrettably,



meticulous matching of central and research council support for academic research is less important than opportunities for diversity. Earmarking is a poor cause.

But that will mean that less is spent on academic research. That will be the quick objection. In reality, however, it need not be like that. For one thing, the proportion of grants committee funds at present notionally devoted to research support is an uncertain figure, dependent on how academics choose to spend their time. The cash component of it is only small (but there is a case for letting universities compete for the annual equipment grant, in round figures about £100 million a year, on the basis of demonstrated need). If self-governing universities choose to spend less of their time on scholarship and more on teaching, this element of the dual-support system for research will vanish in any case. But will that not mean that some universities opt out of research as an integral part of academic life? Perhaps, but institutions cannot rigidly condition how academics spend their time and energy, and Britain is geographically well-shaped (with universities often cheek-by-jowl) for people to pursue their scholarship outside the walls of the institutions that employ them. Indeed, a further erosion of the dual-support system along these lines would only encourage a degree of mobility among academics (once the most mobile of all professional people) sadly absent in the past few years. And to make up for what may at first sight seem to be an undermining of the research enterprise, there is at least a chance that British universities will benefit from the coming reorganization of the research council establishments.

These are the simple questions in the grants committee questionnaire. The British system of higher education includes two kinds of institutions, universities and (as university academics like to think) the rest, mostly institutions called polytechnics. Such admissions statistics as there are suggest that universities have the first claim on young people's interest and that a polytechnic education is chiefly sought either by those to whom university entrance seems unattainable or those who have failed to enter a university. But this is only half the truth. Many of these institutions have academic departments as strong as or even stronger than corresponding departments in universities. And all of them have powerful political support (and municipal loyalty) from local authorities.

So what should be done about the great divide between the universities and the rest of the binary system? Why not simply abolish it? For if the outcome of the latest upheaval in the university system is more diversity and more incentives thereto, the notion that there should be two quite separate parts of the higher education system will become even more absurd than it is at present. And while the problem of simplifying the system as it stands by, for example, merging institutions of the two kinds is always likely to be impeded by academic snobbery and suspicion, the difficulties of merging the rules under which the two kinds of institutions coexist and compete with each other are surely much less formidable. Whether the polytechnics would in present circumstances welcome the chance to be called universities is another matter, while there would be legal difficulties in arranging that those with a consistent academic record should be allowed to award their own degrees. But there seems no point in looking for ways of bridging this snobbish division in British higher education when it is far simpler to get rid of it. That is what the government should be pushing for. □

Who knows science?

The Royal Society Committee studying public understanding should not blame the press.

Do people in general have a sufficient understanding of what science is about and, if not, what can be done to help them? This, improbable as it may seem, is one of the questions the Royal Society (of London) has set out to answer. In reality, the question has not popped up out of the blue, but has been indirectly wished on the society by developments elsewhere, for example by another

of the Royal Society's committees (on education) last year and then, more recently, by the British Government's declaration of interest at the Williamsburg summit in an international programme in scientific understanding. The Royal Society is wise to plan ahead, and to be prepared for the inevitable occasion that will arise when the government turns to it for help with a curious commitment undertaken without much forethought in the heady preparation for Williamsburg. In any case, the committee that will carry out the study (whose chairman is Dr Walter Bodmer, director of the Imperial Cancer Research Fund's laboratories) is a level-headed group, and will no doubt have useful things to say. The danger, as always, is that it will spend its time looking in the wrong places for explanations of irrelevant phenomena.

Very little prescience will be needed to conclude that the public understanding of science (which term must be taken to include technology) is appalling, and probably worse in Britain than elsewhere. One fashionable explanation (in Britain) is that the newspapers serve science badly, which is probably true, and one frequently suggested remedy is that newspapers should be urged (or encouraged) to change their ways, which overlooks the simple truth that the British press is a free press, that the commercial environment in which it survives (sometimes, only just) provides every incentive to give readers what they are likely to want and that the common complaints against the newspapers (not merely for their inadequate treatment of science but for sensationalism, invasions of privacy and the like) mistake symptoms for causes. It is as good a working hypothesis as any other that the treatment of science in the British press is less than satisfactory because readers fail to ask for better. Moreover (as UNESCO — the United Nations Educational, Scientific and Cultural Organization — in another connection is finding to its cost), to seek even by indirect pressure to make a free press behave differently is to put its freedom in hazard. Exhortation and encouragement are, of course, a different matter.

So where else should the Bodmer committee look for a point at which remedies might be applied? It could do worse than begin with the British educational system, and at the secondary schools in particular. But what can be wrong there? Is not the British school system renowned throughout the world for its skill at teaching more specialized science to more students at a more tender age than in any other comparable community? Those who doubt the truth of this claim should look at the examination papers offered to British school-leavers competing for places at British universities (see p.219). The other side of that coin, of course, is that equal numbers of young people emerge from their school careers with an equally specialized knowledge of some quite different field, Latin and Greek for example, and with only a smattering of science gleaned from some early experience of some specialized science course. The plain truth is that in Britain from the age of thirteen hardly anybody is offered help in coming to grips with science in general. Even the courses offered to people aged 13-16 under the headings of "physics", "chemistry" and so on are more like preparations for the more rigorous specializations of the school-leaving examinations than educational experiences in their own right. Moreover, British universities (with one exception) provide no relief from these trammelled educational careers. Is it any wonder that most scientists in Britain end up with few skills outside their own special fields but with a resentment that those who administer their affairs (such as far too many civil servants) are ignorant of what laboratories are for?

But, the Bodmer committee will protest, how can it be possible to advocate a radical reform of British secondary education (and of university admissions procedures as well) at a time like this, when there is no money to spare? The climate may, however, be more favourable than it appears. The British Government has for some time been preaching the importance of a core curriculum for secondary schools, and seems to have been making headway. And when higher education is (yet again) in a state of flux (see above), the right word from a potentially influential committee such as Bodmer's might persuade some of them that the time has come to give up their insistence on school specialization. Certainly no harm can come from telling the truth. □

Three Mile Island

Utility indicted for criminal misconduct

Washington

THE Metropolitan Edison Company has been indicted on eleven counts of criminal misconduct after a three-year investigation by a federal grand jury into the operation of the Three Mile Island power plant; the jury concluded that for months before the accident at the plant in March 1979, the company had been deliberately falsifying the results of safety tests on the crucial water coolant system.

The grand jury's investigation began after a former reactor operator at Three Mile Island, Mr Harold Hartman, alleged that before the accident, Metropolitan Edison falsified results of safety tests to avoid shutting down the reactor in accordance with the safety procedures specified by the Nuclear Regulatory Commission (NRC). The indictment confirms Hartman's allegations and charges Metropolitan Edison with "a pattern of criminal violations" beginning some time in October 1978 and continuing until the day of the accident.

At the heart of the indictments is a charge that Metropolitan Edison deliberately manipulated its water inventory balance tests, commonly known as the leak rate test. Under NRC regulations, the amount of reactor coolant leaking from the Three Mile Island reactor from unidentified sources should never have exceeded a gallon a minute. If it did, the company had either to reduce leakage back to the allowable level within four hours or reduce power and shut the reactor within 36 hours.

The grand jury claims that Metropolitan Edison knew in 1978 that the leak rate test it was using was an inaccurate measure of the amount of coolant in the reactor, but continued to use "a meaningless procedure" to generate results that appeared to establish that leakage was within acceptable limits. In the course of the tests, the company added water and hydrogen to the coolant system in a deliberate attempt to manipulate the results.

Other indictments say the company failed to reduce power when leakage exceeded the allowable rate, that it destroyed records of the test results despite a regulation that they should be retained for at least five years and that it concealed from NRC the fact that the leak rate test being used was not an accurate measure of coolant leaks.

The maximum total fine for all the violations is \$85,000 and the cost of the prosecution — considerably less damaging to Metropolitan Edison's finances than to its reputation and that of the nuclear industry as a whole. Department of Justice officials refuse to say whether the grand jury

believed the company's violations contributed to the 1979 accident, but the indictment is bound to fuel public speculation on those lines. The accident was caused by a failure of the reactor's pressurizing system and the consequent reduction of coolant level in the reactor and overheating of the uranium fuel.

For the General Public Utilities (GPU) Corporation, Metropolitan Edison's parent company and the organization responsible for cleaning up Three Mile Island, the grand jury indictment is merely the latest in a series of unwelcome developments that have forced the company onto the defensive and clouded its ability to raise the \$1,000 million it needs to complete clean-up operations by 1988 as planned.

GPU's financial predicament is made worse because NRC has not yet given the company permission to restart the undamaged Unit 1 reactor at Three Mile Island. Still at issue, in NRC's view, is whether the company management has the competence and integrity necessary for operating a nuclear reactor. NRC's doubts concern not only the implications of the Hartman allegations, but also allegations that the company broke NRC regulations during the clean-up operation and failed to submit two consultants' reports promptly to NRC.

In September, an interim report released by NRC upheld allegations that many NRC

rules and procedures had been violated during the clean-up operation at Unit 2 (see *Nature* 6 October, p.461). The commission is also investigating charges that employees who complained about the rulebreaking were later intimidated and victimized. In June, NRC commissioner Victor Gilinsky issued a scathing memorandum outlining allegations of mismanagement at the company and warning that he would not approve a restart at Unit 1 until GPU's top managers were replaced.

GPU, meanwhile, has gone to extraordinary lengths to rebut those allegations. At the company's request, Admiral Hyman Rickover, former head of nuclear propulsion for the US Navy, is making an independent appraisal of the company's management abilities. The company has also produced a point-by-point response to the Gilinsky memorandum and has commissioned an independent assessment of the clean-up operation by Edwin Stier, former head of New Jersey's criminal justice division. Because the proceedings of grand jury inquiries are secret, the company has not yet been able to respond to last week's indictment.

Meanwhile, the clean-up operation at Three Mile Island is slowing down because GPU is short of funds. Entries to the containment building, once running at the rate of one a day, are down to one a week. According to a spokesman, some \$400 million of the total \$1,000 million set aside for clean-up has already been spent. The cost is being shared by GPU ratepayers, the utility industry, the federal government and the states of Pennsylvania and New Jersey. The utilities, however, have not yet stumped up their "voluntary contribution" of \$150 million. **Peter David**

Argentina to cut nuclear programme?

VICE-Admiral Carlos Castro Madero has announced his "irrevocable" decision to resign as head of Argentina's National Atomic Energy Commission (CNEA) — at the same time, giving strong hints that the new government of Raul Alfonsín may have to make major cutbacks in the existing nuclear energy programme.

Since the early 1950s, Argentina's nuclear power programme has been aimed at self-sufficiency in the production of fuel and heavy water as well as in the operation of power stations. In all negotiations with nuclear equipment suppliers, notably West Germany, Switzerland and the Soviet Union, CNEA has always tried to obtain technology which it could operate without foreign aid. Argentina has its own uranium deposits, and as late as 1980, CNEA officials were speaking confidently of fuel self-sufficiency by mid-1982, when the Córdoba enrichment plant was due to begin operation — to be followed by heavy water self-sufficiency in 1983 with the completion of the Aroviito plant.

It is not clear whether these plants are yet

in operation. Announcing his resignation on 4 November, Castro Madero told the press that the current nuclear plan up to the year 2000 included the construction of four power stations and "feasibility studies" of fuel and heavy water facilities, without making it clear whether these latter included Córdoba and Aroviito. Implementing these plans, he admitted, would involve "obvious financial difficulties" to the new government.

The vice-admiral's departure from CNEA raises once again the possibility of Argentina's signing the Non-Proliferation Treaty. Castro Madero has on several occasions clashed with the "London Club" of nuclear suppliers over their "negative attitude" towards countries that do not yet have their own nuclear bomb. In 1980, he publicly boasted that "with a little help" Argentina would be close to making her own nuclear bomb. Last April he commissioned a feasibility study on the production of nuclear-powered submarines for the Argentine navy.

Vera Rich

European molecular biology

Laboratory awaits British decision*Heidelberg*

THE European Molecular Biology Laboratory, still awaiting to see whether the British Medical Research Council (MRC) will continue its membership, now expects next week's council meeting to pass off without a final decision. The expectation is that the British delegation will abstain (as it did last year) in voting on the budget for 1984, and that a final decision on continued British membership will not be known until after a meeting of MRC in December.

The issue is nevertheless crucial for the future of the laboratory, not merely because of the size of the British contribution (14.7 per cent of the total this year but destined to rise to 16.7 per cent next year) but because of the possibility that other members might follow a British lead in pulling out. (The Netherlands is thought to be an unsure member, for example.)

British reconsideration of its membership stems from a sentence in last year's report of the Advisory Board for the Research Councils which recommends that the question should be raised. Since then, MRC has told the governing council what is afoot, has appointed a review group to make recommendations (thought to be favourable) on future membership, but still has to receive opinions from its internal committees, its Cell Board in particular.

Dr Lennart Philipson, the Swedish director-general of the laboratory for the past two years, is in the circumstances as much occupied with nail-biting over administration as with the scientific programme. The laboratory is vulnerable to the criticism of its members because of its relatively high cost, but Philipson insists that it cannot be accused of extravagance in its running expenses, costing no more than comparable laboratories elsewhere (and likely to cost less in future, with the introduction of a new system for controlling consumable expenses at the beginning of 1984). The salaries of staff members are the chief bugbear, having originally been modelled on those enjoyed at the European Organization for Nuclear Research (CERN), but Philipson also argues that it is appropriate that people on short-term contracts should be compensated for the inevitable insecurity of their positions as well as for the laboratory's denial of outside consultancies.

Conditions of employment at the laboratory seem to be a constant headache. The total staff complement has been fixed at 255 (including 120 working scientists), leaving only five vacancies as things are. At any time, the scientific population may be half as much again because of visitors on "soft" money. The issue of tenure remains a problem. The council has accepted the principle of rolling tenure (on a three or five year basis) but has also set up a com-

mittee to decide how many posts should qualify under this heading. Meanwhile, visiting postdoctoral fellows are unhappy with a scheme for equalizing stipends now that the fall of the deutschemark relative to the US dollar requires "levelling down" and not the opposite.

Philipson's difficulty in these circumstances is that of attracting able groups of people. But many of the laboratory's member countries insist that its proper role should in any case be to provide a place at which their nationals can acquire skills which are then transferred back again. The snag is that such a recipe cannot work if everybody is a short-term visitor.

Philipson's chief initiative so far has been to import (from the Deutsches Krebsforschungszentrum) a team concerned with differentiation in haematopoiesis under Dr Thomas Graf, now established at the laboratory with a staff of twenty and a dozen doctoral students and postdoctoral fellows. Attempts to repeat this pattern by recruiting people skilled at the manipulation of yeast genes have so far been unsuccessful, chiefly because potential can-

didates have usually been offered better jobs in their own institutions. But the search continues.

For the rest, the laboratory is finding the management of its DNA sequence bank more difficult than expected. Like other centres (such as Los Alamos, in the United States) it appears to have found that the mechanical task of storing the data now being published is relatively straightforward, and that the intellectual difficulty lies in writing computer programs intelligent enough to answer the kinds of questions potential users have at the backs of their minds. This section of the laboratory is likely to grow, as is that concerned with the amino acid sequencing of femtomole quantities of proteins.

What happens if the British eventually say no remains to be seen. Philipson points out that the British contribution to the laboratory is less than the total payment (by way of salaries and allowances) to British nationals, but this is unlikely to carry much weight with the British Treasury or the Medical Research Council. In any case, the laboratory disdains the operation of a national quota system, and is free to appoint people of any nationality to its staff, scientific and otherwise.

John Maddox

Soviet science awards**A long time coming**

THIS year's Soviet State Prizes, according to Academician Anatolli P. Aleksandrov, president of the Soviet Academy of Sciences and head of the Council of Ministers' Committee for Lenin and State prizes in Science and Technology, were awarded on the criterion of contributing to the technological and industrial progress of the country. That science should so contribute has been a major theme in Soviet planning during the past two decades, but this is the first time that the relation between prizes and production has been stated so clearly. Commenting on the awards in *Pravda*, Aleksandrov said that at a time when "the threat to peace is growing" and "attempts are being made" to slow down the "progress" of the Soviet Union by forcing it to divert resources from the national economy to defence, it has become vitally important that Soviet industry and technology should be made more effective and productive.

Since there is a notorious timelag between new discoveries and their implementation this criterion has meant that fourteen out of the seventeen prizes in the science section went to large teams (often from several different institutions) for work carried out over a long period of time. Several prizes go to such "cycles of works" originating from the 1960s, one goes back to 1954 and one — for the wave-dynamics of gas-liquid systems — covers thirty years (1952–82). The only three research projects not dated in this way deal with the autogeneration of a light-wave

front in conditions of forced scatter on ultrasound, a study of the human chromosome in normal and pathological conditions and research into amyloidosis. A study of mobile genes which won a state prize for Georgii P. Georgiev and nine collaborators is dated "1972–81", starting two years before the big Soviet drive in molecular biology was launched.

The same emphasis on long-term work is evident in the prizes for textbooks. All four works honoured were revised editions, including a ninth edition of Ivan M. Vinogradov's *Principles of Number Theory* for universities and a 14th edition of N. A. Maksimov's *Physical Geography* for fifthgrade school-children.

The 27 prizes in the "technology" division, however, concentrated on new technologies — pride of place going to Anfiya A. Baulina and her team whose new process for producing chromium compounds has brought in bulk orders from more than 30 countries. The borderline between "science", "technology" and "textbooks" seems blurred. This year the technology section includes two awards for innovations which serve not industry but research — "the development of photographic materials for astronomy, spectroscopy and the diagnostics of thermonuclear laser plasma" and "the development and production of marked tritium compounds for physicochemical biology and biotechnology".

Vera Rich

Chemical arms control

Destruction of weapons on show

Washington

THE United States this week put on a show of "verifiable" destruction of chemical weapons for members and observers of the 40-nation Committee on Disarmament, in an effort to rally support behind its negotiating position at the Geneva talks on chemical arms control. The demonstration, at the Tooele Army depot near Salt Lake City, in Utah, was designed to convince the parties to the talks that sufficient technical means are available for monitoring and verifying the destruction of chemical weapon stockpiles — provided they are supplemented by the continuous presence of on-site inspectors.

The United States and the Soviet Union have, from the outset of the negotiations, been at loggerheads on the issue of verification. Unlike existing chemical weapons conventions, which in effect ban only the first use of the weapons in war, the treaty under negotiation at Geneva would also outlaw the production or ownership of the weapons. Until last year, the Soviets had rejected all proposals for on-site inspections to verify existing stockpiles and their destruction. The Soviets have since eased their stance, but continue to oppose anything more than a limited number of periodic inspections.

The facility at Tooele Army Depot is an automated "disassembly" line that remotely dismantles a shell, removes the chemical agent and incinerates it. Scales and analytical instruments at various points along the line measure the amount of nerve agent destroyed and verify its chemical identity. The plant is currently being used by the US Army to destroy deteriorating munitions in the US stockpile.

US officials said they hoped to persuade the delegates that although instrumentation can confirm that what is supposedly being destroyed is indeed being destroyed, the instruments themselves are not enough. Robert Mikulak of the Arms Control and Disarmament Agency (ACDA) argued that without a human presence to monitor the destruction, it is impossible to tell if someone is "putting his thumb on the scale" or otherwise tricking the instruments. Instruments also need to be recalibrated regularly and the inevitable false alarms must be investigated. Without permanent on-site inspectors, a false alarm would necessitate shutting down the facility until the inspectors arrived. Under a proposal tabled by the United States at Geneva in July, the inspectors would be an international team, similar to that which carries out the enforcement of nuclear safeguards for the International Atomic Energy Agency.

The Soviet Union declined to attend the demonstration. But Romania, Yugoslavia and the People's Republic of China sent delegates, as did 26 other members and

observers of the Committee on Disarmament.

According to former ACDA negotiator Charles Flowerree, the Soviets may have declined the invitation in part to avoid being placed in the position of having to reciprocate and in part to avoid endorsing what they see as a US propaganda stunt. Flowerree added that the Soviets may well have felt that they already know everything they need to know about the plant, which is normally off limits and under tight security.

The attention given this week to the verification of destruction of existing stockpiles seemed to some observers disproportionate to the role that issue is currently playing in the negotiations. The much more difficult issues of verifying the numbers of weapons both sides have to

begin with have been neglected. At a briefing for reporters last week, Colonel Hal Brown of ACDA admitted that this will be a "monumental task", and that no ideas for tackling this problem had even been tabled so far.

Meanwhile, the Senate and the House of Representatives are approaching a showdown over whether production of chemical weapons should be resumed after a hiatus of 14 years. The administration has strongly supported a request for funds to begin production of binary chemical weapons, which it claims are needed to replace deteriorating stockpiles of older weapons and to maintain a "credible" deterrent. Last week, the Senate, with a casting vote by Vice-President George Bush, approved funds for the new weapons. The House, however, which has refused to appropriate any funds, is thought to have the edge in the House-Senate conference that will resolve the matter.

Stephen Budiansky

Thermonuclear fusion

Materials collaboration planned

EUROPE and Japan will join in a \$200-million United States experiment to test materials for a fusion reactor, the Fusion Materials Irradiation Test Facility (FMIT), if recommendations by an expert panel of the International Energy Agency (IEA) are accepted. The panel, whose chairman is Sir Alan Cottrell, the British metallurgist, claims in a technical report to IEA that FMIT — or something like it — is necessary to the long-term development of fusion power.

FMIT would produce an intense beam of 14 MeV neutrons by stripping a deuteron beam in a cascade of molten lithium. These neutrons would mimic and even accelerate damage caused by neutrons from a working fusion reactor — which will displace every atom in an exposed material "some tens" of times a year. According to a US Department of Energy spokesman (and the Cottrell panel), existing irradiation facilities are too weak in intensity and thus too slow in causing damage to do the job. FMIT could compress five years of reactor operation into two years, it is claimed. The research is necessary because the materials damage will be greater in a fusion than in a fission reactor, and will involve some transmutation into gaseous elements such as hydrogen and helium, which might enhance embrittlement. In the long run, it might be necessary to design new materials before a fusion reactor could work economically, or at all.

The US Department of Energy (DOE) has already spent \$80 million on research and development and design work for FMIT, and is now ready to begin construction — if it can find foreign partners to share the remaining \$120 million. DOE sought those partners through IEA in Paris, and IEA sought advice from Sir Alan's panel. Since that advice is positive,

the matter now moves into a more political arena: can and should Europe and Japan (the main members of IEA besides the United States) pay? Japan, it is said in Washington, is ready to pay — but not unless Europe joins in. But Europe would probably participate through the Euratom programme, which is managed by the European Commission and embroiled in the apparently irresolvable budgetary conflicts of the whole European Economic Community. Euratom pays a net 45 per cent of the total \$290 million annual European fusion research budget (including the Joint European Torus (JET) at Culham), and coordinates all the national programmes; and it is currently planning its new five-year programme (1985-89), for submission to the European Council of Ministers in July.

"If we contribute to FMIT, it should be in this five-year period", said a Euratom spokesman on Monday, "but the 'if' should be in capital letters."

If Europe does not come up with the goods, FMIT would seem to be doomed — but only for the time being. According to DOE, the research and design work could be mothballed, to be called out when necessary. The next generation of reactors — after JET and similar machines — can be designed without the aid of results from FMIT, but the generation after that — something close to a demonstration reactor — would need to feed in FMIT know-how. Design work on such reactors might begin in the late 1980s or early 1990s. FMIT is designed to make studies quickly, but if it proves that new materials will be necessary, as it might, then its data would be needed earlier than if all went smoothly. Such questions are now under consideration in Washington. By contrast, Brussels is "dragging its feet".

Robert Walgate

Hungarian universities

Degrees of difference

HUNGARY'S system of postgraduate education has been reformed. Last month, the Presidential Council approved a law establishing a new scientific degree, that of "university doctor", ranking below the existing degrees of "candidate" and "doctor of sciences". Ironically, the change has been urged by academics as a way of making young scientists less diploma-orientated.

Under the old system, young scholars could not even begin work on their candidate dissertation until they had published several pieces of research. One result is that of Hungary's 50,000 "scientific workers" (including social scientists and humanists), only some 20 per cent have a higher degree. Under the new system, however, people will be able to apply for a special "university fellowship" immediately after taking their first degrees. Fellowships will be awarded on the basis of an examination that will include a foreign language and a "philosophical-ideological" section as well as the applicant's special subject, and will carry a stipend of 5,200 forints a month, comparable with a university lecturer's salary of 5,800 forints.

The completed dissertation will be defended after three years before a committee of both academy and university specialists. Those submitting dissertations of a sufficiently high standard will be awarded a candidate's degree, while the less outstanding but still competent will receive the lower degree of "university doctor".

Although the new procedure will become law only in September 1984, the first batch of applications was accepted last December, and 390 fellowships have already been awarded (out of 520 applicants). These include not only 1982 graduates but also several young scientists who did not yet have the necessary publications to start a candidate's dissertation under the old system. A number of young physicians were also attracted to the new fellowships, as a means of switching from practical medicine to medical research.

Although it is too soon for the scheme to be assessed, a preliminary report presented to the presidium of the Academy of Sciences (which has overall responsibility for the operation of the scheme) suggests that the initial selection procedures are working satisfactorily, with 50 per cent of the new fellows accommodated in university research facilities and the rest in academy institutes. The chance of a higher degree at an earlier age may help to overcome the dissatisfaction caused by the extremely low starting salaries of young graduates, often below those of a manual worker of the same age.

Vera Rich

European Science Foundation

Fund of ideas but no funds

Strasbourg

AMBITION and timidity again collided at this year's general assembly of the European Science Foundation, Europe's unique blend of grant-making organization and learned academy whose members appoint themselves. Yet paradoxically, the foundation seems in good spirits.

Last week, the assembly was told of a plan to mount an infrared telescope in a converted civil aircraft (to fashion an "astroplane") and of the lingering hope (after four years of frustration) that European governments will find a way to build a super electron storage ring for generating synchrotron radiation. But the foundation also relinquished direct responsibility for one of its successes so far — a fellowship and training programme in neurophysiology — and was told clearly that it would lose all credibility with social scientists if it could not raise the funds needed for a study begun three years ago of language learning among adult immigrants.

The foundation's difficulties mostly arise because it is an impresario for research, not a research organization as such. Of next year's budget of FF8.95 million (£734,000) (approved last week), close on 90 per cent will be spent on administration — always vulnerable, never popular. And although the foundation will probably handle as much again on account of its research projects (quaintly called "additional activities"), that part of the budget is contributed only by those members willing to support individual projects.

Whence the row about language learning. The objective is to understand how adult immigrants — Bengalis and Italians in Britain, Finns in Sweden, Turks in Germany and Moroccans in France, for example — acquire the language of their host environment (if at all). The project fits in well with others of the social science projects, including a major study of intra-migration due to be completed next year. Unfortunately, when the foundation willed the end, it was constitutionally incapable of willing the means.

Professor W.J.M. Levelt, director of the Max-Planck-Institut für Psycholinguistik at Nijmegen (Netherlands), which coordinates the project, said last week that work was well under way on the core of the project — a scheme to assess the linguistic progress of a sample of more than 200 guest workers in five different countries. Yet last year (1982), the foundation had wrung only FF1.4 million from its members towards the total cost of FF2.4 million. The Max-Planck-Gesellschaft had bridged the gap, paying the salaries of four research workers and even supplying the five research teams with a minicomputer for use

in data analysis.

But this cannot carry on, and is in any case "not acceptable", Levelt said. "You cannot vote year after year to approve the budget and then fail to accept the consequences of your joint decision." And if the further FF8 million that will be needed (over three years) had not been promised by the end of the year, activities would have to be curtailed and "the reputation of the European Science Foundation with social scientists will be in question". Most members looked at their feet, but the Finnish Academy and Norway promised to look at the question urgently, and Britain and France said they would consider increasing their contributions.

These are the hazards ahead of the astroplane. According to Professor C. de Jager of the Space Research Laboratory at Utrecht (Netherlands), the objective is to modify either an Airbus or a Canadian Challenger aircraft to carry 2-m and 1-m infrared telescopes respectively. Total costs, including 300 hours of operations a year over the decade, would amount to FF350 million for the Airbus and FF150 million for the Challenger. De Jager argued that the project would be complementary to satellite and ground-based observations, but that it would put balloons out of the infrared business. It seems to be understood that France and Germany will decide between them which option, if any, flies.

The European synchrotron radiation project is in much the same position. Refinement has continued of the design for a 5-GeV storage ring capable of generating synchrotron radiation at a wavelength of 0.1 Å. There are now several potential sites all over Europe, but none of the host governments has as yet offered a sufficiently large share to get the project started. Meanwhile, the planners are worried by the proposal of Stanford University in California to build a 6-GeV storage ring for the same purpose. The general opinion is that a decision must be made by next year or the European project must be abandoned.

Technically, this is what has happened to the European Training Programme in Brain and Behaviour Research, which organizes training courses and provides student travel grants in fields related to neurobiology. Having decided a year ago that the programme should be left to its own devices so as to free itself for new initiatives, the foundation was this year faced with a demand from the sponsors that it should continue to administer the scheme, which will henceforth be designated an "associated programme".

Professor H. Curien, the foundation's president (who is head of the Centre National d'Etudes Spatiales in Paris), explained that the underlying objective is to let successful projects make their own

way in the world so as to concentrate the foundation's energies on new projects. The Max-Planck-Gesellschaft seems to have been especially influential in this decision but observers (not all journalists) wonder why the foundation should discard the feathers in its cap, especially when its members are unready to turn their enthusiasm for innovations into cash.

None of this seems to have prevented the assembly last week from pushing ahead with other plans. Byzantine and Chinese studies flourish, and the deep seismic sounding project known as the European Geotraverse has begun well. (There are even plans to interest the Soviet Union in the inclusion of the Kola Peninsula.) The foundation is also helping geophysicists to press their claims for a fair crack of the whip in the launching of European satellites, its long-standing interest in polymer science has led to the planned formation of a European Polymer Foundation, and the foundation programme of fellowships in toxicology has spawned an agreement with the European Commission for a study of the toxicology of chlorophenol chloropropenes designed to provide Brussels with a scientific basis for regulation, and intended by both parties as a proving ground for Community regulations.

The formation of a consortium to allow the foundation's smaller member states to take part in the continuation of the US Deep Sea Drilling Project due to begin next October is more of a scramble. Professor R. van Lieshout, director of the Netherlands science research council (ZWO) and organizer of the consortium, explained that the rush stems from the late decision on the future of the deep-sea drilling in the United States. But now "the train has already left the station".

There seems no prospect that the smaller members of the foundation will be able to raise the \$2.5 million a year required of full members, so that hopes turn on whether Britain will opt for full participation (a decision still to be made by the UK Natural Environment Research Council), as France and West Germany have already done. One parsimonious solution canvassed last week is that the smaller members might club together to pay the \$200,000 needed for the first year's membership, thus influencing the programme, but then decide to pull out. Final bids will be disclosed at a meeting in Zurich early in December.

For the rest, the assembly approved the launching of a project on forest ecosystems after (and in spite of) an impassioned speech by Professor W. Bossard, director of the Swiss Federal Institute of Forestry Research, threatening catastrophe for alpine valleys from avalanches and soil erosion if forest die-back were allowed to continue. The assembly also elected Professor van Lieshout and Professor T.D. Spearman (Trinity College, Dublin) as vice-presidents for the next three years.

John Maddox

UK research staff

Too many dead-end jobs?

THE conditions of employment of British university research workers, many of whom are engaged on short-term contracts, represent the "unacceptable face of the high-tech revolution", a meeting of the Association of University Teachers (AUT) was told last week. Mr John Akker, deputy general secretary of the association, said that researchers' terms and conditions of employment were more like those of farm workers than of key workers in a vital industry.

AUT estimates there are 12,000 research staff employed in the universities, including those in research council units. Their contracts are normally attached to particular projects and may last anything from 3 months to 5 years. Almost all contract researchers, according to AUT, are required as a condition of their employment to sign a waiver clause denying them the right to claim unfair dismissal or to seek a redundancy payment. Dr B. Salter, who chaired an AUT advisory group on the subject, says that this clause contravenes the spirit of the relevant legislation, which was intended to apply to well-paid short-term contracts. Researchers are paid less than lecturers and advance more slowly.

Because conditions of service and opportunities for advancement are unattractive, few contract researchers stay for long: in 1980, 81 per cent had been researching for less than 5 years, although their academic qualifications were similar to or better than those of lecturers. Mr Akker last week described the treatment of researchers as a "wasteful and gross exploitation of talent". He said that the Prime Minister should examine the problem urgently if she wishes to increase output from science and technology.

The meeting, attended by 90 delegates representing all of Britain's universities, formally established a National Committee of Contract Researchers. The committee will hold local meetings throughout the country and will seek meetings with the University Grants Committee, the Committee of Vice-Chancellors and Principals and research councils, as well as government. AUT intends to make full use of legal sanctions where these are applicable and may consider demonstrative action.

The main aim is to establish a recognized career structure. AUT will ask universities to consider the establishment of "bridging pools" to pay researchers between projects, funded by means of a surcharge on research contracts. But AUT also wants to see other improvements in researchers' terms of service.

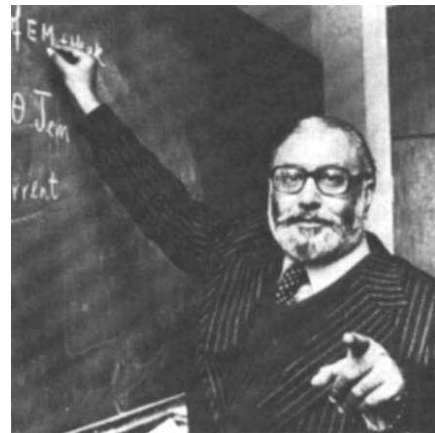
It was pointed out last week that many lecturers, on accepting early retirement, receive golden handshakes of £20,000 or more. A researcher of the same age would receive nothing.

Dr Salter, who is now a member of the contract researchers' committee, is under no illusions about the difficulty of the task AUT faces. One problem is that responsibilities are fragmented: most researchers are paid by universities, which in turn receive grants from research councils. Neither will tackle the problem without the other, says Salter. Another difficulty is that a young research population is often thought to be a good source of fresh ideas. Salter dismisses this notion, and argues that the same might be said of any profession.

Tim Beardsley

Developing academy

PROFESSOR Abdus Salam, the Pakistani physicist and champion of Third World scientists, is to be president of a "Third-World academy" of leading scientists in and from the developing world, it was announced last Friday at a ceremony in Trieste, Italy. The academy — which, says Salam, "will be happy to advise governments and other bodies" on matters of science and development — begins with 25 full fellows, two of them Nobel Prize winners, chosen from the existing fellowships of prestigious academies including the US National Academy of Sciences, and 14



Salam presides

associate fellows. The associates are ex-nationals of developing countries now working in the West.

The academy will have four main roles, Salam said last week: to support "good men" in developing countries; to advise; to publish a newsletter on scientific issues in the Third World (to be edited in Caracas); and to promote "South-South" collaboration in science.

The academy will be "a collection of individuals", and thus a totally different exercise from the UN University, which Salam condemns as too bureaucratic. However, the new academy has as yet no budget. Salam sees himself doing a lot of the planning and writing of letters. The money will come, he says. Robert Walgate

Technology transfer

High-tech for Britain

A NEW company specializing in technology transfer was launched in Britain last week. The company, Business Application of Science and Technology — BASE — aims to take an active managerial role in seeking new applications for research results and also to become a source of investment capital.

The chairman of BASE International is Sir Henry Chilver, vice-chancellor of Cranfield Institute of Technology and chairman of the government's Advisory Council on Applied Research and Development (ACARD). BASE is developing a network of contacts in universities and government and corporate laboratories and will assess technical and commercial opportunities for innovation. Where opportunities are identified, BASE will buy or obtain under licence technologies to place with its client companies. Financial returns will be from royalties and, in some cases, capital growth.

By seeking to introduce new technologies to existing companies, BASE hopes to avoid some of the problems that beset would-be innovators. It aims to bridge the "development gap" that has been identified in a number of studies of innovation in Britain, not least those of ACARD. BASE says it will acquire technologies from a wide range of sources and will where necessary sponsor further research and development to match them to market requirements.

BASE starts life with a modest £450,000 of capital, subscribed mainly by the Royal Life Insurance Group, TR Technology Investment Trust and the BP Pension Fund. The Cranfield Institute of Technology is also an investor, through its own research and development company. BASE's managing director, Mr John Castle, has a personal stake of £20,000. There are plans to double the capital base as the company grows and BASE will seek additional capital from its shareholders as it is required, on a project-by-project basis.

BASE will certainly benefit from the government's decision to rescind the first option on much publicly-funded research that is now enjoyed by the British Technology Group. BASE will have no preferential access to publicly-funded research. The company has already identified a number of projects in which it might wish to invest, and Sir Henry said last week that the civil application of defence technologies was a area of interest. One project BASE is examining was originally developed at the Royal Signals and Radar Establishment.

BASE looks on the face of things like a textbook model for innovation, and the connection with ACARD is very apparent in BASE's approach. The low uptake of defence technology has been criticized widely in recent years and defence

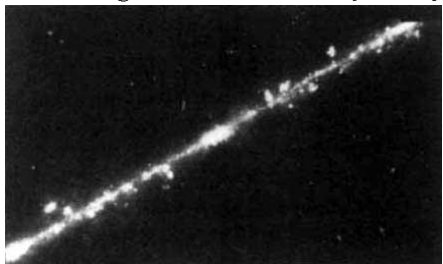
establishments are thought to be fertile fields for entrepreneurs. Last September, Mr Michael Heseltine, Secretary of State for Defence, announced that he was inviting technology brokers into defence establishments to examine technologies with possible commercial applications, and through Sir Henry's connection with Cranfield, BASE will have a direct route to a substantial pool of university engineering research. Industry sources were impressed last week by the account BASE gave of itself.

Tim Beardsley

Galactic centre seen

THE first results from the Infrared Astronomical Satellite (IRAS) were announced last week at simultaneous press conferences in the United States, the Netherlands and the United Kingdom — the three countries running the mission.

The photograph below shows IRAS's view of 45 degrees of the galactic plane, including the galactic centre, completely obscured at optical wavelengths by interstellar dust. The bulge is the galactic centre, while the knots and blobs are giant clouds of gas and dust heated by nearby



stars. The data confirm that star formation is taking place — the gravitational contraction that precedes the onset of energy generation by nuclear reactions releases heat, so that proto-stars are strong infrared sources.

Other newly discovered objects include three continuous dust rings within the Solar System, a broad central band and two rings 9° above and below it, lying between Mars and Jupiter at a distance of 2.3 to 3.3 astronomical units. One theory for their origin is that a comet, with an orbital inclination of 9° to the plane of the Solar System, collided with an asteroid. IRAS has also observed many new regions of star formation, a ring of large dust particles around the star Vega — thought to be a proto-solar system — and galaxies in which intense bursts of star-formation are taking place. The latter also appear to be the most likely explanation for unidentified objects — objects for which no previously observed counterpart has been recorded. Such galaxies would produce a great amount of energy in the infrared but could be so far away that the optical radiation is too dim to have been observed.

Philip Campbell

Life in outer space

Infrared data debugged

THE conflict between Professors Sir Fred Hoyle and Chandra Wickramasinghe and their critics, astronomers and spectroscopists sceptical of the claim that life is to be found in interstellar space, was left unresolved last week. A meeting arranged by the Royal Astronomical Society was marred by a lack of time for free discussion. But at the end, none of the protagonists had conceded ground.

By Professor Wickramasinghe's account, he and Hoyle were persuaded of the existence of interstellar microorganisms by the failure (as he described it) of more conventional models of interstellar grains to match features in astronomical spectra. Specifically, the astronomical evidence consists of (1) an infrared absorption feature at 3.4 micrometres wavelength observed in the spectrum of a star (IRS-7) known to be obscured by interstellar dust; (2) features at about 10 micrometres in the thermal radiation spectra from the Trapezium nebula; (3) the variation of the interstellar extinction of ultraviolet radiation with wavelength and, in particular, a claimed feature in this variation at a wavelength of 2,800 Å.

Hoyle and Wickramasinghe have measured the absorption properties of *Escherichia coli* and "microorganisms from a nearby river" and, as reported in several publications, have managed to obtain good fits to the astronomical observations with, they claim, a success far exceeding that of modellers using more conventional materials.

Various aspects of this work were attacked by speakers at the meeting. Thus Professor J. M. Greenberg (University of Leiden) noted that in fitting an amino acid with an excess in ultraviolet extinction at 2,800 Å, Hoyle and Wickramasinghe had overlooked that the spectrum observed from the International Ultraviolet Explorer (IUE) was "over-exposed" and flagged as such. Thus, Greenberg said, the feature is spurious. Hoyle's reply that the level of noise seemed to be consistent across the wavelength band was dismissed by other speakers.

A similar difficulty arose with the infrared feature at 3.4 micrometres. Dr H. Kroton (University of Sussex) emphasized that there is virtually an infinity of ways of producing this feature, since any compounds containing C-H bonds will exhibit absorption in this range. Thus, nobody should consider such a spectral match as unique — and in any case the data used by Hoyle and Wickramasinghe in their match with laboratory absorption measurements of *E. coli* were shifted in wavelength from the original publication. Hoyle and Wickramasinghe responded by saying they had used the data as supplied by the

original investigators, but the discrepancy remained unresolved.

A successful fit to the infrared data based on more conventional materials was provided by Dr D. A. Williams (University of Manchester Institute of Science and Technology), who offered hydrogenated amorphous carbons (HAC) as candidates. They contain carbon both in benzene-ring and in tetrahedral bonds, with hydrogen atoms also present; Williams used them to deny claims for the uniqueness of biological material without claiming that HAC is actually "up there".

To Greenberg's calculation that, given

photochemical processes in grains, bacteria would be destroyed within a few thousand years at the most, Hoyle replied that he and Wickramasinghe did not claim that all the bacteria in space are alive, but that colonies of bacteria might enjoy a degree of protection in the nuclei of comets.

Briefly, the meeting allowed participants the opportunity to state their positions for and against the idea that life exists in the interstellar medium. Many direct questions were left in the air for lack of time. An hour of unplanned debate might have done everybody involved a great deal of good.

Philip Campbell

Paranormal weapons

Congress flirts with psychic war

Washington

THE Soviet Union is making "significant progress" on military uses of psychic phenomena, according to a US Army study which recommends that the United States should begin work on defensive measures. The report, disclosed in a new book on "psychic warfare"*, seems certain to find at least a few receptive ears on Capitol Hill. Earlier this year, several legislators commissioned a study by the Congressional Research Service (CRS), an arm of the Library of Congress, on the status and potential of "psi" research, and the resulting document, no doubt reflecting the congressmen's hopes, is remarkably optimistic.

After summarizing the "relatively consistent informal replication" of psi phenomena, which include extrasensory perception, psychokinesis, miscellaneous apparitions, out-of-body experiences, and reincarnations, the CRS report offers some "conservative" speculation on possible applications. Besides the rather mundane possibilities in animal training and finding lost wallets, the report notes that "remote perception, precognition, and retrocognition are of potential interest to intelligence agencies, law enforcement units, and activities involving surveillance". The report also warns that "low-level of micro-PK" effects might cause malfunctions in microelectronic circuits, and that before sensitive equipment becomes even more dependent on microchips, the phenomenon should be better understood.

The Soviet Union, CRS says, has already shown interest in dowsing for water, oil and minerals (dowsing is taught to professional mineralogists at Tomsk Polytechnic Institute). The Soviets are also widely suspected of investing tens of millions of roubles in psi research directed at military applications such as "remote hypnotic manipulation".

The US Government, by contrast, supported only four psi research projects, totalling \$179,500 between 1971 and 1978. The largest source of funding for such research in the United States is a private group, the McDonnell Foundation,

which donated \$500,000 to Washington University to establish a psychic research laboratory. That laboratory last year became one of the many victims of James "the amazing" Randi, a professional magician who specializes in exposing psychic research. Randi sent two of his protégés to amaze the researchers with their "psychic" abilities at spoon bending and other tricks, and then revealed at a press conference their true identities — after the researchers had published several papers on the subjects.

The CRS report dismisses such sceptics in a few paragraphs, noting that "the quality of criticism of psi research is often poor" and that polls of both the general public and the scientific community show a "generally positive attitude" towards psi research.



A CRS staff member refused to identify the congressmen who commissioned the study; the report itself, however, notes that a workshop on the subject conducted by the "Congressional Clearinghouse on the Future" elicited considerable interest on the part of "certain members and their staffs".

Senator Claiborne Pell (Democrat, Rhode Island) who is known to support psychic research (although a staff member, perhaps trying to play down the issue, attributed the senator's interest to the eclectic mind of a "renaissance man"), was quoted last week by the Associated Press as saying, "I personally have never experienced or seen a psychic event, but it seems to me that there have been adequate scientific articles written that would indicate that they do occur".

Stephen Budiansky

**Psychic Warfare: Threat or Illusion?*, Martin Ebon.

UK higher education

Oxbridge scraps the old rules

THE universities of Oxford and Cambridge have now introduced changes in their admissions procedures intended to increase the proportion of their undergraduates coming from British state schools.

Traditionally, applicants for Oxford and Cambridge have taken a special entrance examination peculiar to those universities, but in recent years more places have been awarded on the basis of performance in school-leaving A-level examinations. In both universities, about half the applicants for places have attended independent fee-paying schools, as have about half of those who are successful, although only five per cent of sixth-formers attend such schools.

The more radical of the changes agreed are at Oxford, where the undergraduate colleges have decided after a long and difficult review that candidates applying to the university after their school-leaving examinations should not in future sit the entrance examination. Candidates applying before finishing school study will retain the option of taking the examination. The practice of allowing post-A-level candidates to compete against others with less experience has been widely criticized, not least because few state schools are able to provide post-A-level coaching. This may be one reason why many state schools do not at present encourage applications to Oxford or Cambridge.

But the Oxford colleges failed to agree on a recommendation from Sir Kenneth Dover, chairman of the committee set up to review the admissions procedure, that pre-A-level candidates should be free to choose whether or not to sit the entrance examination. Some admissions tutors are reluctant to admit undergraduates without having seen their performance in the examination, and they have insisted on the right to advertise their preferred method of entry in particular subjects.

In another move which will increase the incentive for state school applicants, both Oxford and Cambridge have decided to stop giving awards to candidates who excel in the entrance examination. This feature of the admissions system has been criticized for encouraging premature specialization and, again, for seeming to favour candidates from independent schools, which are better able to provide the sort of special tuition likely to result in awards.

For the time being, Cambridge intends to keep the post-A-level entrance examination. If the two universities go their separate ways, with only Cambridge allowing post-A-level examination candidates, some independent school candidates may see Cambridge as the better bet: only there will they have the opportunity to make amends for disappointing school results.

Tim Beardsley

Scientists quiet in Japan

SIR — You suggest in your special issue on "Science in Japan" (*Nature* 29 September) that the answer to the "cry" for more creativity in Japan lies simply in substantial support of basic science in the universities (p.382). In 1972 you complained of the relative neglect of basic science by the Japanese Government (p.356); this year you indicate again that "more autonomy, more resources for research and more say in how they are used" remain as a remedy for the present state of universities in Japan. You are afraid that "tradition and the government's budget officers would be offended, but those would be small prices to pay for an overdue reform" (p.382).

Scientists in universities are a potential source of criticism of the policies of a very conservative government. By keeping the universities short of money in comparison with booming industry, and providing ample research grants to a selected few conformist scientists, the government could effectively control a possibly critical group of scientists while maintaining scientific activity at a reasonable level.

In such an ambience scientists have been rather easily tamed. By contrast, during the post-war period, Japanese scientists could be more independent of government control as illustrated by the early successful activities of the Japan Science Council (p.361); also during the 1960s when large overseas research grants (mainly from the United States) were readily available for internationally active Japanese scientists. This last pattern was soon criticized, however, during the emergence of student power in the late sixties, which was followed by the "oil shock". Then the Japanese economy finally overtook Western rivals, leading to a perfect control of scientists by the government. Both the current detachment of scientists from the once popular Japan Science Council, and the lagging of Japanese biotechnology in spite of the presence of some active groups of molecular biologists (p.377) may be explained by this fact. In genetic engineering, Japanese scientists, as well as bureaucrats, did not like to stand out conspicuously in pushing controversial technology until the "dust had settled". Hence the initially very stringent Japanese guidelines for recombinant DNA were issued only in 1979 instead of 1976, though relaxed quickly afterwards. Public debate on scientific matters is not popular in Japan. I have not seen the "Erato" subjects (p.373) debated extensively in the scientific media, either before they received massive governmental support, or after. Certainly this is not the best way to encourage creativity among scientists and universities. This point is recognized as a dilemma for Japan's quest for creativity in Japan itself as well as in the United States (Keyworth, G.A., *Science* 217, 606–609; 1982).

The following facts would further corroborate my contention. For quite a few years staff members of the national universities, like all other employees of the government, have had to apply for governmental permission for all *private* travel overseas, including holidays. So far, no complaint has been filed from the university members. Unlike Japanese writers and medical professionals the government-dependent Japanese scientists have so far not organized a body working for the cause of nuclear disarmament, while SANA (Scientists Against Nuclear Arms) membership scores nearly 1,000, 340, and more than 100 in the United Kingdom, Australia and New Zealand, respectively. That trend is worrying, because Japanese society now looks alarmingly like that preceding the Second World War when practically all the liberals were effectively silenced. Those who have memories of that period are now reaching retiring age in the leading national universities. Would a conservative government like to boost scientists in the Japanese universities with additional resources now? Powerful opposition parties could promise that, but there is not even one of those in Japan.

ATUHIRO SIBATANI

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Homologyana

SIR — You are not fond of phrases such as "B-cell lymphoma DNA sequence non-homology" (*Nature* 6 October, p.477). In fact, the word homology itself has become the object of a considerable amount of abuse in the columns of *Nature* and of nearly all journals that deal with comparisons of polynucleotide or polypeptide sequences.

Molecular biology has taken over the term homology from biology. According to the biological — and the initial molecular-biological — meaning of the word, two structures are said to be homologous when their similarities are extensive enough for us to assume their derivation from a common ancestral structure. The concept of homology is therefore all-or-none. DNA sequences, RNA sequences and protein sequences are homologous or not. They cannot display strong or weak homology, just as no one can be said to be 65 per cent pregnant (an example furnished to me by Walter Fitch). These remarks do not apply to a special case: two informational macromolecules may be homologous over 65 per cent of their length when the remaining 35 per cent are of a different evolutionary origin.

If another Greek or Latin word were to be adopted in order properly to express the idea that molecular biologists have usually in mind when they speak of 65 per cent

homology, it might be "isology", as proposed many years ago by Marcel Florkin. Meanwhile, why not use the ordinary word "similarity" or "matches"? Two polynucleotide or polypeptide chains can be said to show 65 per cent matches, or a 65 per cent similarity. Using an ordinary word correctly is more professional than using a professional word incorrectly.

Because poor usage becomes good usage as soon as it is generally accepted, advocates of good usage are born losers, unless they fight early and hard. In regard to fighting for a proper use of the term homology, the hour is late.

EMILE ZUCKERKANDL

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Terminal guidance

SIR — With reference to your leading article on 13 October (p.561), all the proposed new Western weapons will have the novel and dangerous property of terminal guidance: so this is a new step for the qualitative arms race. Cruise and Pershing II will be ten times as accurate as SS20s and far more effective for use as war-fighting weapons, or in the case of Pershing II for a first strike.

An analogy from the history of the arms race is the development of MIRV missiles. Not only did this greatly increase the numbers of warheads but, since one missile could then theoretically destroy several on the ground, a disarming first strike became at least a conceivable strategy. Since the potential to retaliate is the basis of mutual deterrence, MIRVing actually decreased our security. Terminal guidance is the next step, the increased accuracy making the theoretical destruction an achievable goal.

ROBERT WALL

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Not fit

SIR — I regret that I am unable to agree with Pirie's assessment (*Nature* 20 October, p.664) that American English is more rational than that written in England, at least on the evidence given. Pirie quotes Gould as writing "this view fit nicely with most nineteenth century concepts," and interprets this use as different from the English norm, which would require *fits*. A simpler interpretation of Gould's reported usage is that *fit* is the past of the verb to fit, not the third person present singular. *Fit* as a past form is given as a second choice in *Webster's Third New International Dictionary*, and agrees with American usage. *Fit* as a third person present singular is not standard in the United States, not even in California.

W.E. HAZEN

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Great greenhouse in the sky?

The latest report on the carbon dioxide problem deserves careful reading but does not settle the question whether there will be calamity. But its proposals for research should be taken up.

WHAT is to be made of the problem of accumulating carbon dioxide in the atmosphere? The latest opinion on the subject, the report of Dr William Nierenberg's committee of the National Research Council of the US National Academy of Sciences, *Changing Climate*, published last month (see *Nature*, 27 October, p. 751) can be read in two ways. Either, innocent readers may conclude, the problem is even more complicated than had been thought, or the new document serves chiefly to confirm the suspicion that there will at some stage be a pronounced alteration of the Earth's climate caused by the greenhouse effect. Especially because the document says nothing new about the climatic models used to predict the changes that may occur, but instead endorses the conclusions of a report published last year under the same auspices by a panel headed by Joseph Smagorinski (*Carbon Dioxide and Climate: A Second Assessment*, Washington DC, 1982), some readers will conclude that the only certainty is that the carbon dioxide problem, whatever it is, is one year nearer than it was a year ago.

That would do the Nierenberg document an injustice. The outstanding issues about the climatic models will not in any case be quickly cleared up. One of these is the difficulty of including realistic clouds in climate models even though it is accepted that clouds must to some extent work towards climatic stability, with higher surface temperatures implying more atmospheric moisture, more cloudiness and thus less radiation reaching the surface of the Earth. Another is the persisting uncertainty about what happens to the carbon dioxide added annually to the atmosphere by the discharge of combustion gases. The Nierenberg report shows more precisely than its predecessors that only a proportion, perhaps 60 per cent, of the annual load of carbon dioxide remains in the atmosphere, so that the rest must disappear either into the oceans or into vegetation. The snag is that there is still no conclusive evidence of how this fraction is related to the total concentration in the atmosphere and so no certain way of moderating future estimates of carbon dioxide concentration by a realistic estimate of the rate at which it will be naturally removed. (If anthropogenic emissions were to cease, the atmospheric concentration would presumably begin to edge back to what it used to be in the early nineteenth century.) On the third major uncertainty in the

climatic models, the still imprecisely known effect of heat (and gas) transfer between the atmosphere and the oceans, however, the new report is informative.

What follows is a miscellaneous selection of some of the data buried in the Nierenberg report, not the least arresting of which is still the direct demonstration, by means of infrared observations of the Sahara Desert from the Nimbus 4 satellite, that carbon dioxide does virtually block out transmission of infrared radiation in the wide band around a wavelength of 15 μm (Hanel, R.A. *et al. J. geophys. Res.* 11, 2629–2641; 1972). Second, there is now ample evidence not merely of the increase of the average annual concentration of carbon dioxide at stations such as Mauna Loa in Hawaii but of the slow increase in the amplitude of seasonal fluctuations of carbon dioxide concentrations (probably caused by the annual growth of vegetation in the spring and summer in the Northern Hemisphere) of about 10 per cent per decade. The accumulation of further data from several stations throughout the world should make it possible to disentangle the causes of the well-known annual fluctuations of carbon dioxide concentration, not nearly as well linked with the calculated emission of carbon dioxide as might be hoped. In his contribution to the Nierenberg report, Lester Mehta even invokes the El Niño phenomenon, the geophysicists' equivalent of the universal solvent, as a possible explanation.

The prediction of future carbon dioxide emissions is probably the best-known part of the whole enterprise, but involves assumptions of two kinds — estimates of future energy consumption, which in turn entails estimating the total level of economic activity, and estimates of the extent to which this will be partitioned between the consumption of fuels releasing carbon dioxide to the atmosphere and other sources of energy, of which nuclear and solar energy are the only significant candidates. The data given in the Nierenberg report argue for the emission of some 10 gigatonnes of carbon dioxide a year by the end of the century (with a factor of two covering the spread of the various estimates). The uncertainties beyond that are, however, so great that a greenhouse effect expected, on present trends, to become apparent only halfway through the next century might in fact not become apparent for a century after that. But provided that the greenhouse effect is not largely cancell-

ed out by some hitherto unknown negative feedback, continued accumulation of carbon dioxide must make the effect apparent later if not sooner.

This is why attention necessarily centres on the oceans, and on the exchange of heat and gases at the interface. The Nierenberg report includes a clear summary by P. G. Brewer of the chemistry of carbon dioxide in seawater, in particular of the mechanism by which it is expected that continuing solution of carbon dioxide, by decreasing the normal alkalinity of saline seawater, will decrease the solubility of carbon dioxide at the surface. This is without question a positive (or destabilizing) feedback, but too little is known of the variations of carbon dioxide removal at various parts of the ocean surface for accurate predictions of the future course of events to be possible. But Brewer does quote evidence to show that the partial pressure of seawater in the Sargasso Sea has been increasing during the past quarter of a century.

Heat removal is a more difficult problem, at least while so little is known of the stability of the oceanic surface layer in conditions very different from the present (such as would obtain if the thermocline were uniformly deeper). But Roger Revell raises in the Nierenberg document a scary possibility — that increased oceanic temperatures might release large quantities of methane from the hydrous clathrates in which it is supposed to be combined in oceanic sediments at great pressure. Methane is, of course, another potential cause of greenhouse warming.

In the most provoking section of the Nierenberg report, G. Weller *et al.* consider the problem of detection. Trends of mean air temperature are shown to be poor indicators of climatic change but also essential — they are the only variables for which long records are available. Sensibly, the group argues for a more comprehensive programme of climatic monitoring if only so that those concerned can evaluate claims to have detected warming. Other possible causes of climatic fluctuation than carbon dioxide, such as other greenhouse gases, volcanic aerosols and changes of solar output, need careful watching, while the group gives priority among climatic variables to the measurement of sea surface temperatures, stratospheric temperatures, variables that might throw light on the radiation balance at the surface of the Earth and the water content of the atmosphere.

John Maddox

Huntington's disease

The beginning of the end of a dilemma?

from Miranda Robertson

AT 4-8 cases in every 100,000 people, Huntington's disease is very rare. But it is among the most mysterious and at the same time peculiarly distressing of all hereditary diseases. It is a degenerative disorder affecting specific regions of the brain, usually first manifesting in middle age with minor movement disorders that progressively worsen, and developing into severe disability and dementia ending in death after 10-20 years. The mystery is how the Huntington's disease gene can cause the programmed death of a specific subset of brain cells. The peculiar nastiness of the disease lies in its late onset and autosomal dominant mode of inheritance. This combination means that any individual with one affected parent has a 50 per cent chance of having inherited the disease — and thus of passing it on to his or her own children — but no way of knowing whether the gene is in fact present until the symptoms appear, often well after the usual child-bearing age. The discovery, reported in this issue of *Nature* (p.234), of a closely linked genetic marker for the Huntington's disease gene may in the short term radically change the predicament facing such individuals; and in the long term it may lead to an answer to the fundamental question of how the disease is caused.

The genetic marker was identified by Gusella and his colleagues through the use of techniques that enabled them to get round the problem of tracing the inheritance of a genetic defect whose molecular basis is quite unknown. Where the molecular defect is known — as, for example, in the case of the hereditary anaemias caused by mutant haemoglobin molecules — the defective gene itself can now be identified by examination of the DNA so that affected pregnancies can be identified and terminated early. The alternative, where the gene itself cannot be identified, is to find a marker in the DNA that is so close to the gene that it is almost always inherited with it. This became possible in principle with the discovery of what are known as restriction fragment length polymorphisms. These are simply slight variations in the DNA of different individuals that cause it to fall into fragments of different sizes when cleaved by bacterial enzymes. When such a polymorphic region of DNA is closely linked to a particular gene, the inheritance of the gene can be traced by following the inheritance of a particular pattern of restriction fragments. The technique is entirely empirical and the polymorphism need have nothing to do with the genetic defect to which it happens to be linked.

Gusella *et al.* have now discovered such a

polymorphic region in close linkage to the gene for Huntington's disease. This means in principle that they can now hope to identify affected individuals before the onset of symptoms; and to detect fetuses carrying the gene early in pregnancy. In practice however it will be some time before such screening will be available, for reasons that can best be illustrated by considering in more detail exactly what has so far been achieved.

The linkage was established through the investigation of two families, one in North America and one in Venezuela, on whom clinical and genetic data have been collected in a massive collaborative effort over three years. To find an appropriate polymorphic marker, Gusella and his co-workers expected to have to work through some hundreds of DNA sequences taken from a library of human DNA, testing each for an association with diagnosed Huntington's disease. In fact, remarkably, they found their marker, a sequence known as G8, within the first dozen they tested. G8 falls into four different patterns of fragments (technically known as haplotypes) which Gusella *et al.* have designated A, B, C and D. In the North American family the A haplotype is associated with Huntington's disease, while in the Venezuelan family the association is with the C haplotype.

This illustrates the important point that the G8 haplotypes themselves are not diagnostic of Huntington's disease: all four of them are present in varying frequencies in the normal population. Thus in order to predict the risk of a given individual's developing the disease, it is necessary to know not only which haplotype the gene is linked with in his or her particular family, but also which haplotypes are present in both parents. Clearly (for example) a member of the Venezuelan family is extremely unlikely to develop the disease if he does not inherit the C haplotype from either parent; but if the C haplotype is present in the DNA of the unaffected as well as the affected parent, or if the affected parent is homozygous for the C haplotype but not for the Huntington's disease gene, the offspring will have no way of knowing whether any C-haplotype DNA he inherits is linked with a lethal or an innocent gene.

As it happens, the C haplotype is relatively rare (it is present in about 20 per cent of the population), so that the probability of its turning up in an unaffected individual is reasonably low and the predictive value of the association correspondingly high. The A haplotype, by contrast, is present in roughly 60 per cent of the population and its predictive value in the North American

family is thus much less.

At present, then, the possibility of screening is limited to those two families and even then has predictive value only for some individuals. It is in the potential of the G8 marker for further studies that its real importance lies. Having established that it is closely linked to the Huntington's disease gene (now mapped to the short arm of chromosome 4), Gusella and his associates can extend their investigations both to other families (this is already under way) and to other surrounding DNA regions. By identifying further polymorphisms they can hope eventually to be able to detect affected individuals with virtual certainty, given adequate pedigree data.

This would, moreover, open up the possibility in principle of eliminating the disease completely in one generation. This is possible because, unlike the commoner hereditary diseases, Huntington's disease seems very seldom to arise anew. There are very few known cases in which an affected relative cannot be traced, and many of the known families can be traced back to a handful of individuals. It is believed, for example, that many cases in New England may originate in East Anglian immigrants in the John Winthrop fleet, while in South Africa the source is apparently a Dutch immigrant and in Venezuela a German sailor. (Clearly further linkage analysis will help to establish just how few the sources actually are.)

In practice however the abolition of the disease, even in several generations, is most unlikely. Surveys of people at risk have shown that at least half would choose not to know if they were going to develop the disease; and there is no doubt that for some affected individuals neither contraception nor abortion would be acceptable as a way of avoiding producing affected children. But those who would prefer to know, and to whom abortion would be acceptable, can realistically expect to be offered a new option: that of knowing for certain whether they will develop the disease; and if so, of having a family without transmitting it to their offspring. For half of those at risk, of course, this would mean the end of all anxiety, either for themselves or for their children.

The most exciting prospect of all however is that the G8 marker will make it possible in the foreseeable future to identify the Huntington's disease gene itself. Because the linkage is so close, further analysis of the DNA in the vicinity of G8 may reveal a polymorphism directly diagnostic of the mutant gene. Once that has been done, the path lies open not only to an understanding of the mechanism of that neurological disease, but to the fundamental questions of how the nervous system develops and — alas — deteriorates with age. □

Miranda Robertson is associate editor of *Nature*.

Particle physics

The F meson found at Cornell

from J. M. Yelton

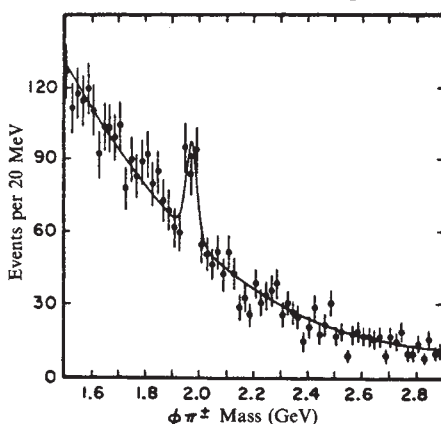
PARTICLE physicists using the CLEO detector at Cornell¹ have recently shown the most compelling evidence yet of the existence of the F^+ meson, the combination of a charm quark with a strange anti-quark². They measure the invariant mass of the F^+ to be $1970 \text{ MeV}/c^2$, filling an important gap in physicists knowledge of charmed mesons.

The discovery of the J/ψ in 1974 started a new era of particle physics and its identification as a bound state of a charm quark with a charm anti-quark was seen as a validation of the quark model of hadronic matter. The model then predicted a whole spectrum of mesons containing charm quarks. Combination of charm quarks with up and down anti-quarks, the D^0 and D^+ , were found in 1975, and their excited states, the D^{*0} and D^{*+} followed soon after. However, the F^+ meson, the combination of a charm quark with a strange anti-quark, has proved difficult to detect. The properties of F^+ mesons may be predicted by analogy with D mesons. The F mass is expected to be somewhat greater than that of the D^+ ($1,868 \text{ MeV}/c^2$), and its decay products to be typically an η or a ϕ meson together with a number of pions. The CLEO collaboration has sought for, and found, a resonance that decays into $\phi\pi^+$ and which they identify with the F^+ meson.

The CESR e^+e^- collider at Cornell produces collision energies of around 10 GeV, just sufficient to create mesons including bottom quarks, but also fairly well matched for studies of charmed mesons. The CLEO detector is a general purpose detector operating at CESR, and incorporates good charged particle momentum measurement. The first stage in their analysis is to make the invariant mass combination of oppositely charged tracks under the hypothesis that they are kaons (tracks clearly identified as pions are excluded). A clear signal corresponding to the ϕ meson (mass = $1,020 \text{ MeV}/c^2$) is seen. Those combinations with mass within $5 \text{ MeV}/c^2$ of the ϕ mass were taken as ϕ candidates. Combinations were then made of the ϕ candidates with each remaining pion in the event. The invariant mass spectrum for $\phi\pi^+$ combinations with momentum greater than $2.5 \text{ GeV}/c^2$ is shown in the figure. A clear peak is seen, with a width consistent with that due to experimental resolution alone and an invariant mass measured to be $1970 \pm 5 \text{ MeV}/c^2$, where the errors are statistical and systematic respectively. The probability that the peak is due to a statistical fluctuation alone is around 1 in 20,000, and its decay characteristics are consistent with it being an F^+ . Although confirmation of

the signal in another channel (for example, $F^+ \rightarrow \phi\pi^+\pi^+$ would be very welcome, there seems little doubt that the resonance observed by CLEO is, indeed, an F^+ meson.

Previous experiments have shown some evidence for the existence of F^+ , produced



by a variety of mechanisms and decaying into an η and pions^{3,4}, and also into a ϕ and a ρ^+ (ref. 5). Although the mass measurement from each individual experiment was weak they tended to indicate an F^+ mass of around $2,030 \text{ MeV}/c^2$, so the CLEO result has come as something of a surprise. Theoretically, the exact value of the F^+ mass is interesting as it helps constrain models of the shape of the potential force

binding together the quark and anti-quark. In principle, from a knowledge of this binding force together with a face value for the effective masses of the various flavours of quark, it should be possible to calculate the masses of all mesons. This is made more difficult by the fact that in the charmed mesons the quark masses are small compared with the kinetic energy of the quarks inside the mesons, so relativistic effects are important.

The CLEO experiment will not be able to measure the lifetime of the F^+ produced, but several experiments using optical techniques (bubble chambers and nuclear emulsions) have already tried⁴. Their main problem is unambiguously separating the F^+ from other charmed particles. The F^+ lifetime is expected to be similar to that of the D^0 (around $4 \times 10^{-13} \text{ s}$), but there is still some controversy as to the exact decay mechanism of charmed mesons and a precise measurement of the F^+ lifetimes should help clarify the situation.

At a time when there are big projects being undertaken to create e^+e^- collisions of energies around 100 GeV, the CLEO result highlights the amount of physics still to be explored at lower mass scales. □

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1. A. Chen *et al.* *Phys. Rev. Lett.* **51**, (1983). The CLEO group is a collaboration from the Universities of Cornell, Harvard, Ohio, Rochester, Rutgers, Syracuse and Vanderbilt, and Ithaca College.
2. Reference to a state will always imply the sum of that state and its charge conjugate state.
3. R. Ammar *et al.* *Phys. Lett.* **94B**, 118 (1979).
4. R. Brandelik *et al.* *Phys. Lett.* **80B**, 412 (1979). D. Aston *et al.* *Phys. Lett.* **100B**, 91 (1981).
5. D. Aston *et al.* *Nucl. Phys.* **B189** 205. (1981).

Human genetics

Human gene mapping rolls along

from Ellen Solomon and Peter Goodfellow

THE enormous task of mapping the human genome was begun in 1936 using the classical Mendelian techniques of family and population linkage studies. This approach eventually ran into difficulties because there were not enough polymorphic markers available, and in 1970, it gave way to new methods using somatic cell genetics. The random loss of human chromosomes from human/rodent somatic cell hybrids made it possible to map genes by correlating the presence of human genes, or gene products, with particular human chromosomes. In addition, the large genetic differences between humans and rodents meant that many more genes could be studied. By 1980 the technique had allowed approximately

300 genes to be assigned to particular autosomes — compared with only 20 in the previous 50 years.

Now the new decade has contributed a new revolution: gene cloning. Paradoxically this revolution has completed the circle; the application of DNA probes and associated restriction enzyme site polymorphisms (also known as RFLP for restriction fragment length polymorphisms) can provide an unlimited number of genetic markers for studying human families. Mendelian genetics is once again the major tool for mapping the human genome. Future historians of science will have no difficulty in discerning these trends as they are illustrated by biannual meetings devoted to human gene mapping. The latest* was held in California and was the first fully to reflect the resurgence of the old genetics.

The short-term goal of human gene

*Human Gene Mapping 7, to be published by the March of Dimes and Cytogenetics and Cell Genetics. Organised by R. Sparkes, M.A. Spence and T. Mohandas in August, 1983, and held at U.C.L.A.

mapping is to cover the genome with polymorphic chromosome-specific markers (either defined genes or arbitrary DNA sequences) at regularly spaced intervals. If these markers are sufficiently polymorphic and close enough together finding a linked marker and thereby mapping any single gene trait or inherited disease should be straightforward. Using the linked markers, family studies and genetic counselling should become feasible for the whole range of disorders where biochemistry has as yet yielded nothing amenable to heterozygote detection, amniocentesis or therapy. The approach has proved its value for the X-linked Duchenne muscular dystrophy² and has also shown that Becker muscular dystrophy maps close to or is allelic with Duchenne muscular dystrophy (H.M. Kingston, University of Wales, Cardiff).

Since the last workshop³ there has been a six-fold increase in the number of DNA probes described. These include 115 cloned genes (of which 32 have a defined associated polymorphism) and 142 arbitrary RFLP. Unfortunately we are still some way from covering the whole genome and more markers, especially those which are highly polymorphic, are needed. For example, it was calculated that for a dominant disorder a linkage study of 15 families, with six children (each) and their parents using classical markers, such as isozymes and red blood cell antigens, will cover only eight per cent of the genome. This number increases to 44 per cent with the currently available RFLP (M. Scolnick, University of Utah and H.F. Willard, University of Toronto).

Without waiting for complete genome coverage several groups have used the new markers for linkage analysis. The most successful and exciting result is the discovery, reported in this issue of *Nature* (see p.234 and *News & Views* p.222), that Huntington's Disease is tightly linked to an RFLP which itself has been provisionally assigned to chromosome 4 (J.G. Gusella, Harvard University). The isolation of more closely linked polymorphic probes in this region may enable genetic counselling for this dominant disorder to begin.

Also receiving renewed attention are the non-random chromosome rearrangements found in malignancies. These, of course, reflect the chromosomal localization of the cellular *onc* genes (Table 1); most striking being the position of *c-myc* at the 8/14 breakpoint in Burkitt's lymphoma (reviewed in ref.4) and *c-abl* near the 9/22 breakpoint in Ph¹ positive CML⁵. A major effort of the next few years will be to examine closely the non-random rearrangements in haematological malignancies for proximity to cellular *onc* genes.

The solid tumours, with few exceptions, are much more difficult to approach in this manner. Analysable chromosome preparations from such tumours are always difficult to obtain, and where they are available they generally show gross

Provisional table of oncogene localisation

c-onc	Chromosome
n-ras	1p31.1-p22.1
sk1	1q12-qter
fos	2
raf-1	3
raf-2	4
fms	5q34
myb	6q15-qter
rasK-1	6p32-q12
erb B	7pter-q22
mos	8q22
myc	8q24
abl	9q34
rasH-1	11p15.1-pter
rasK-2	12p12-pter
fes	15q22-q26
erb A1	17p11-q21
src	20
sis	22q11-qter
rasH-2	X

aneuploidy and multiple rearrangements, rather than a consistent pattern. Here, the availability of RFLP probes as markers for each chromosome is of enormous advantage and genetic analysis of the tumour compared with the tissue from which it is derived, is possible. Exciting results using this type of approach have been found with retinoblastoma and have very recently been reported in *Nature* (see Cavenee *et al.* *Nature* 305, 779 and *News & Views* 305, 761). Using RFLP probes on chromosome 13, constitutional and

tumour material from these patients was compared. In several cases markers which were heterozygous in the constitutional tissue became homozygous or hemizygous in the tumour. The mechanisms generating these results were gross chromosomal changes involving chromosome loss, chromosome loss plus reduplication, or mitotic recombination. (W.F. Cavenee, University of Utah School of Medicine).

RFLP and chromosome-specific probes are currently selected from libraries made from somatic cell hybrids containing a single human chromosome or from flow-sorted chromosomes. The latter technique is highly specialised and is only being carried out in a few laboratories at the moment. A collaborative effort between Lawrence Livermore Laboratory and Los Alamos has been funded to make chromosome-specific libraries generally available. Requests for further information and for specific chromosome libraries can be made to Dr A. Carrano at the Lawrence Livermore Laboratory in California. □

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1. Bell, J. & Haldane, J.B.S. *Proc. R. Soc. B* 123, 119 (1936).
2. Davies, K. *et al.* *Nucl. Acid. Res.* 11, 2303 (1983).
3. *Human Gene Mapping*, 6 *Cytogenet. & Cell Genet.* 32, 1 (1982).
4. Klein, G. *Cell* 32, 311 (1983).
5. de Klein, A. *et al.* *Nature* 300, 765 (1982).

Phylogenetics

Origins of the lower invertebrates

from Robert D. Barnes

A recent symposium* on the origins and relationships of lower invertebrates took a fresh look at the evolutionary origin of the Animal Kingdom and the phylogenetic positions of animal phyla and their relationship to the pathways of early animal evolution. Most of the new work appearing in this area reflects the great contribution of ultrastructural work to our knowledge of lower invertebrates and the growing use of cladistic analysis in determining phylogenetic relationships from comparative morphological data.

A flagellate ancestor was generally assumed for the metazoans, confirming at least indirectly the 'colonial flagellate theory' and recognizing the primitive nature of the monociliated condition in animals. The choanoflagellates appear to be the most likely protozoan ancestral group (T. Cavalier-Smith, Kings College London; C. Nielsen, Zoological Museum, Copenhagen). The principal area of debate

was the nature and origin of the acoelomate, or solid, body plan. The older view (defended by P. Ax, University of Göttingen) holds that flatworms are primitively acoelomate and thus are near the evolutionary base of the bilateral phyla. Others (R.M. Rieger, University of North Carolina; M.E. Fransen, State University of New York Medical Center at Syracuse), using evidence from ultrastructural studies on turbellarian parenchyma and myoepithelium argued that the acoelomate condition is secondarily derived from a coelomate condition. They cite the lack of uniformity in the parenchyma of different turbellarian groups and that coelomate linings appear to be derived from myoepithelium rather than from mesenchyme.

Papers on other groups of invertebrates proposed some new ideas about invertebrate phylogeny and reaffirmed some older ones. Sponges probably had a common origin with other metazoans and do not warrant their usual separate subkingdom status. The structural peculiarities of the glass, or hexactinellid, sponges, especially their syncytial nature, might, however, justify their removal from the Porifera and being given status as a separate phylum

*The symposium on The Origins and Relationships of Lower Invertebrates was sponsored by the Systematics Association and held at the British Museum (Natural History) on September 7-9, 1983. The proceedings will be published under the editorship of R. Gibson (Liverpool Polytechnic), S. Conway Morris (University of Cambridge), and J.D. George and H.M. Platt (British Museum, Natural History).

(P.R. Bergquist, University of Auckland). Ctenophores are probably not closely related to the cnidarians despite their superficial similarity and long standing alliance with that group (G.R. Harbison, Woods Hole Oceanographic Institution).

The aschelminth phyla appear to belong to two different phylogenetic lines. One embraces the rotifers and acanthocephalans, which have intracellular cuticles, and the other includes the gastrotrichs and nematodes, which have an extracellular cuticle (S. Lorenzen, University of Kiel; P. Clément, University of Lyon; P.J.S. Boaden, University of Belfast).

A new phylum, the Loricifera, was

proposed for a small group of marine, microscopic, interstitial animals superficially similar to kinorhynchans (R.M. Kristensen, University of Copenhagen). The body is surrounded by a lorica, from which the phylum name is derived, and the anterior end bears a retractile spiny introvert with buccal stylets.

The sipunculans retain their position near the base of the annelid-mollusc stem (M.E. Rice, Smithsonian Institution), and the pogonophorans continue to be placed near the annelids (M.L. Jones, Smithsonian Institution). □

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Radiation protection

Natural radiation risks

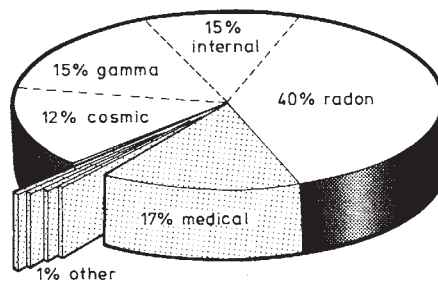
from M.C. O'Riordan

AT a recent conference* on indoor exposure to natural radiation, sources, levels, and effects were assessed. The need was seen to control high doses in homes, but not in a precipitate or fragmentary manner.

The strangest paradox in radiation protection is that the least attention is paid to the strongest sources of human exposure. According to the latest report of the United Nations Scientific Committee on the Effects of Atomic Radiation, the average dose received by people living in industrial countries is about 2.4 mSv a year, of which 82 per cent is from natural radiation, 17 per cent from medical procedures, and 1 per cent from other artificial sources. This last category attracts considerable attention, because it includes occupational exposure to ionising radiation and routine discharges of radioactive materials to the environment by the nuclear power industry. It is no exaggeration to say that public and professional interest in natural and artificial radiation is inversely proportion to the radiation dose received from them.

The natural radiation to which humans are exposed consists of four components — cosmic, gamma, internal, radon. The relative contribution that each makes to the sum is shown in the chart. Cosmic ray exposure and internal exposure of the body from natural radionuclides in diet are virtually unaffected by ordinary circumstances, but exposure of the whole body to terrestrial gamma rays and of the lungs to radon daughters are influenced by the nature and location of housing. Gamma rays are emitted mainly by the trace quantities of uranium and thorium in earth and building materials, and they irradiate the human body more or less

uniformly. Radon isotopes occur in the decay series of these radioelements, and since radon is a gas, it readily emanates from porous materials such as soil and brick in which it is generated. If it emanates out of doors, it is readily dispersed and diluted in the atmosphere: if it emanates indoors, the activity concentration may become quite high because of the restricted supply of outside air. It is not so much radon itself that is of concern, but rather the short-lived daughters of the gas, which are radioactive isotopes of solid elements such as polonium. These solid particles



Components of the average annual dose of 2.4 mSv.

form a radioactive aerosol; when inhaled, they irradiate the lung tissues and increase the risk of lung cancer.

Not much time was spent on gamma rays at the conference. The physics of gamma-ray emission and human irradiation are well understood and the standard risk factor is applicable. (The dose quantity widely used in radiation protection is the effective dose equivalent, expressed in sieverts, symbol Sv. It is a measure of radiation risk rather than a measurable quantity: the standard risk factor for fatal malignancies and equally serious effects is 1.65×10^{-2} per Sv.) Many national authorities have made or are making thorough surveys of indoor gamma exposure. Doses are already fairly well established, with an average value around 0.35 mSv a year and with few

persons receiving more than a few mSv a year. The practical view of the matter is that indoor exposure to gamma rays is unexceptional and unavoidable. Indoor exposure to radon daughters is, however, quite another matter.

There is little doubt that the main source of indoor radon is the ground on which a house stands. In a typical masonry house, about 50 per cent of the dose from radon daughters is due to the ground and 25 per cent each to the structure and the incoming air. For a wooden house, of course, the ground contribution is relatively more important, whereas for a high apartment it is insignificant. In otherwise ordinary houses, therefore, extraordinarily high doses are explicable only in terms of elevated radon ingress through the floors.

Houses would appear to be very efficient radon collectors. The gas is transported to the sub-structure by diffusion and convection and is then literally sucked into the structure through cracks and ducts in the floor. Not enough is yet known about the effect of underpressure indoors to be able to model the transport processes conclusively, but there are already clear pointers to the procedures for preventing high levels. These are to avoid building on sites with high radon emanation, to make the floor as impermeable as possible, and to evacuate the radon from the sub-structure. Any of these is better than removing radon daughters after the gas has got into the house, and there have been some encouraging trials in a few countries.

Another exacting problem is to convert activity concentrations of radon daughters in air to dose. It is necessary first to characterise the indoor aerosol, then to model its deposition and retention in the respiratory tract, next to estimate the radiation energy absorbed by the sensitive cells, and finally to calculate the effective dose equivalent. The problem has been resolved to the extent that there is broad agreement on the value of the conversion coefficient, although there is room for considerable refinement at each stage and some investigators have reservations about the utility of the last stage. Having effected the conversion, one can use the standard risk factor, which, in this case, would be entirely expressed in lung cancers. The outcome of the whole exercise is underpinned by epidemiological data for excess lung cancers among miners exposed to radon daughters with appropriate adjustment for the different circumstances of exposure.

Many national authorities are also conducting substantial surveys of indoor exposure to radon daughters. The results so far available point to an average dose of 1 mSv a year, but unlike gamma-ray doses, the variability is pronounced: many persons receive over 10 mSv a year and some even exceed 100 mSv a year. Annual doses in this range and the implied risks were thought by many participants to be unacceptable and unnecessarily high. At 50 mSv a year continuously, for example, the pro-

*ENEAC-CEC International Seminar on Indoor Exposure to Natural Radiation and Related Risk Assessment, 3-5 October 1983. The proceedings will appear in *Radiation Protection Dosimetry*.

bability of premature death from lung cancer would reach five per cent or so. Little or no concern was expressed about the general shift towards higher doses that results from reducing ventilation rates in homes to save energy — except when it exacerbates existing difficulties.

Some national authorities have already recognised the need to deal with high doses from natural radiation indoors and are carefully considering the social as well as physical aspects of the problem. Others are still at the planning stage of substantial research on indoor pollutants in general and radon daughters in particular. A few,

one suspects, are simply praying that the problem will go away. Most, however, were hoping for strong recommendations from the International Commission on Radiological Protection to prevent the development of conflicting protection schemes throughout the world. This hope will be realised sooner than the participants expected: recommendations were agreed by the Commission within days of the conference and will soon be published. □

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Behavioural ecology

Do frogs and toads choose their mates?

from Tim Halliday

In the last few years, the dense breeding aggregations that frogs and toads form in the spring have been the subject of numerous studies by behavioural ecologists^{1,2}. One of the questions that these studies have sought to answer is whether females actively choose to mate with certain males rather than others. As the evidence accumulates, it is becoming clear that active female choice occurs in only a minority of species and that, in most, observed patterns of mating are largely explicable in terms of interactions between males. Arak's paper on Natterjack toads (*Bufo calamita*), published in this issue of *Nature* (see p.261), is the latest to reach this conclusion.

To demonstrate that females have evolved an adaptive mechanism for choosing particular males, three kinds of evidence are required. First, it must be shown that females do not mate randomly. Second, it is essential to establish that any non-random effect is a consequence of female behaviour and not of competition between males, which is typically very intense in anuran mating systems. Third, to show that female mating patterns are adaptive, and are not merely a consequence of some other process, it must be demonstrated that females who mate with their preferred males have higher fitness. There is abundant evidence that mating in anurans is non-random, rather less that this is due to female choice, and hardly any that female choice is adaptive.

One study that has obtained good evidence for all three aspects of female choice is that by Howard on the American bullfrog (*Rana catesbeiana*)³⁻⁵. Male bullfrogs establish territories in a breeding pond and females show a preference for those defended by the larger males. These territories are optimal for egg development, in that they contain warm water that hastens embryonic development and contain low numbers of a leech that attacks

developing eggs. For many anurans, however, the breeding season is much shorter than it is for bullfrogs and males do not establish territories¹. For these 'explosive breeders', mating involves what appears to be a chaotic scramble in which it is difficult to envisage, let alone demonstrate, any role for female choice.

In explosive breeders, the principal character investigated in relation to whether or not mating is random is body size. There is generally considerable size variation within both sexes and the question is: do females mate preferentially with the larger males, with males roughly the same size as themselves (size-assortative mating), or at random? From studies of the common toad (*Bufo bufo*) in Britain, it is clear that, when animals first arrive at a pond, pairing tends to be random^{6,7}. However, by the time that spawning takes place, a few days later, larger males are significantly more likely than smaller males to be paired with females. This is a consequence of fighting between males, not of female choice⁶. Males greatly outnumber females in toad breeding populations and unpaired males attack and attempt to dislodge those that are in amplexus with females. Small amplexed males are unable to resist the attacks of larger males and, after a prolonged struggle, are displaced. The time between initial pairing and spawning appears to be a crucial factor in determining the extent to which fighting can influence male mating success². In the neotropical toad *Bufo typhonius*, whose breeding activity lasts less than a day, there is no size-related pattern in the distribution of matings among males, except that the very smallest males do not mate. It appears that there is simply not time for fighting to have an influence on the mating success of the majority of males⁸.

An intriguing situation is that presented by species that show size-assortative mating^{9,10}. This appears to be adaptive for

both sexes. Fertilization is external and fertilization success should be maximized when male and female are of the same size, because their cloacas will be very close together¹¹. However, there is not a shred of evidence that males and females 'size each other up' and choose a partner of the same size as themselves. Rather, size-assortative mating seems to be a consequence of male fighting; males who are much larger or much smaller than the females with whom they are amplexed are more likely to be dislodged than those who are well matched and so can maintain a tight grip.

In many anurans, Arak's natterjacks among them, males produce loud calls that attract females. This raises the possibility that females could choose males of a preferred size on the basis of call characters, some of which are correlated with male body size. Most commonly, larger males have calls with a lower fundamental frequency. There is evidence that the body-size information encoded in a male's call is important in determining the outcome of fights between males^{12,13}, but the idea that females respond to such information remains controversial. It has been suggested that females should prefer larger males because these are generally older and have therefore shown their capacity to survive¹⁴.

Arak's study of natterjacks suggests that females do not respond preferentially to lower frequency calls of larger males, but to louder calls, irrespective of their frequency. However, Ryan^{15,16} showed that females of the Central American frog *Physalaemus pustulosus* approach the deeper calls characteristic of the largest males in preference to the high-pitched calls of small males. These results can be criticised, however, on the grounds that the choice presented is between extremes¹⁷. In nature, the majority of males will have intermediate calls and it is necessary to show that females prefer the calls of the largest males to those of average males. Working with spring peepers (*Hyla crucifer*), Doherty and Gerhardt found no preference for the calls of larger males over those of average-sized males¹⁷.

Many anurans share their breeding sites with other species and, in at least one species, this appears to be related to a female preference for the calls of average-sized males. The green treefrog (*Hyla cinerea*) is partly sympatric with the barking treefrog (*H. gratiola*) and the squirrel treefrog (*H. squirella*) whose mating calls have, respectively, slightly lower and slightly higher frequencies than those of *H. cinerea*¹⁸. Female green treefrogs discriminate against calls characteristic of the largest and the smallest conspecific males, thereby reducing the risk of engaging in hybrid matings. So high are the costs of hybrid matings, that it is likely that selection favouring accurate recognition of species will be much more powerful than that favouring preference for one kind of conspecific male over another.

Even if female frogs do choose males on

the basis of their calls, it is not necessarily certain that they will be able to exercise their choice freely. In several species, only some males call at any one time, other, non-calling males adopting a 'satellite' strategy, taking up positions near a calling male so that they can intercept females that approach him^{19,20}. Sullivan has observed the response of female Great Plains toads (*Bufo cognatus*) to satellite males in some detail²¹. As they approach a calling male, females generally try to evade any satellite male that approaches them. If they are grasped by a satellite, they are usually unresponsive and attempt to escape. However, a satellite can successfully secure a female if he grasps her at the moment she makes contact with a calling male. Female avoidance of satellite males is evidence of female choice, though it may well not be the case that it implies avoidance of conspecific males who are, in some sense, inferior to callers. Males who do not call are, from the female's point of view, of unknown species, and so avoidance of satellites may simply be a mechanism for avoiding hybrid matings.

Finally, demonstrating that females respond preferentially to males whose calls have particular properties, such as low frequency or high intensity, does not necessarily imply that such a preference has an adaptive basis. Females may simply be more strongly stimulated by certain kinds of calls or may find them easier to locate in space, because of the physical properties of their auditory system². Such behaviour would be what Parker²² has described as 'passive attraction', in contrast to 'active choice'. Although passive attraction may not confer any benefit on females, it clearly does on males; sexual selection will favour males whose calls possess the properties most effective in attracting females. □

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1. Wells, K.D. *Anim. Behav.* **25**, 666 (1977).
2. Arak, A. in *Mate Choice* (ed. Bateson, P.) 181-210 (Cambridge University Press, 1983).
3. Howard, R.D. *Ecology* **59**, 789 (1978).
4. Howard, R.D. in *Nature Selection and Social Behaviour* (eds Alexander, R.D. & Tinkle, D.) 61-77 (Chiron Press, Norton Mass, 1981).
5. Howard, R.D. *Am. Nat.* **122**, 301 (1983).
6. Davies, N.B. & Halliday, T.R. *Anim. Behav.* **27**, 1253 (1979).
7. Gittins, S.P., Parker, A.G. & Slater, F.M. *J. Nat. His.* **14**, 663 (1980).
8. Wells, K.D. *Biotropica* **11**, 301 (1979).
9. Lee, J.C. & Crump, M.L. *Oecologia* **50**, 153 (1981).
10. The European common frog (*Rana temporaria*) shows size-assortative mating (Personal observation).
11. Davies, N.B. & Halliday, T.R. *Nature* **269**, 56 (1977).
12. Davies, N.B. & Halliday, T.R. *Nature* **274**, 683 (1979).
13. Ramer, J.D., Jensen, T.A. & Hurst, C.J. *Copeia* **1983**, 141 (1983).
14. Halliday, T.R. in *Mate Choice* (ed Bateson, P.) 3-32 (Cambridge University Press, Cambridge 1983).
15. Ryan, M.J. *Science* **209**, 523 (1980).
16. Ryan, M.J. *Evolution* **37**, 261 (1983).
17. Doherty, J.A. & Gerhardt, H.C. *Anim. Behav.* (in the press).
18. Gerhardt, H.C. *Amer. Zool.* **22**, 581 (1982).
19. Perrill, S.A., Gerhardt, H.C. & Daniel, R. *Science* **200**, 1179 (1978).
20. Perrill, S.A., Gerhardt, H.C. & Daniel, R. *Anim. Behav.* **30**, 431 (1982).
21. Sullivan, B.K. *Anim. Behav.* **30**, 939 (1982).
22. Parker, G.A. in *Mate Choice* (ed. Bateson, P.) 141-164 (Cambridge University Press, 1983).

World petroleum congress

Hot prospects in the South China Sea

from Roger N. Anderson

THE talk of the 11th World Petroleum Congress, in London for their 50th Anniversary meeting in September, was of the petroleum prospects along the northern margin of the South China Sea. This southern margin of the People's Republic of China, is the single largest remaining unexplored offshore basin in the world. Industry estimates put the recoverable oil and gas possibly trapped in the massive geological structures beneath the margin at equal to, or greater than, the entire proven reserves of the North Sea¹.

Since the opening of East-West relations by President Nixon in 1973, there has been an intensive effort at exploration by both conventional and unconventional geophysical means. The problem — given that the basin has no previous drilling record — is to estimate just how likely it is that the right processes have occurred in the geological past.

Are there sufficient traps to accumulate the large volumes of oil and gas required to make drilling in remote offshore areas profitable? A geological trap is formed when impermeable or cap rock stops the naturally buoyant flow of oil and gas, both lighter than water, upward toward the surface. Geological forces, which cause continental drift and which push the great plates across the World's surface, deform the subsurface rocks forming faults and folds and force impermeable shale on top of more permeable sandstone. These forces rifted the island of Borneo away from southern China about 30 million years ago, and left behind huge structural traps one to three miles below the surface. These traps can be seen by seismic reflection profiling from a ship steaming over the top of the structures — the conventional method used by the oil industry for discovering new oil and gas deposits. An oil company must be as certain as possible that oil and gas have accumulated in these geological traps before drilling can begin. Eight out of every ten oil wells in unknown territory are dry or unproductive and at twenty million dollars a well, a massive investment is required. The southern China margin will be particularly expensive, mainly because of the tough position taken by the People's Republic of China. Indeed, it will probably be one of the most costly drilling efforts ever launched by the giant international oil companies and profit margins will be quite narrow.

Evaluation of the chances that oil and gas have accumulated requires knowledge of the source rocks. Petroleum is not formed where it is trapped but in organic-rich shales. It must be baked in a geological

'pressure-cooker' before oil (medium heat), then gas (high heat) are formed. The same grade of oil can be cooked quickly at high temperature or slowly at low temperature.

Most gasoline burned in your automobile is 100 to 500 million years old. That is, it was cooked for a very long time at low temperatures. Some oil in the world is very young, however. Offshore California oil, for example, is less than 8 million years old. Why? Because it cooked quickly at high temperatures. But if the oil overcooks, first gas will form instead of oil, then it will sour and nothing useful will be formed.

An unconventional method of geophysical prospecting has been used to evaluate the 'cooking' process south of China. Called thermal maturation analysis, the amount of heat escaping from the surface is measured. Then, using a geological model of the rifting history of the margin from 30 million years ago to the present, the thermal history of the source rocks buried on the South China Margin is evaluated.

What thermal maturation analysis coupled with present day heat flow measured across the continental margin is showing is that the margin is too hot for a pure oil discovery. Much gas is surely present, with the largest amounts of oil to be found in the deepest waters. But at current world prices, it would not be economical to exploit it! But China has a great need for energy. Selling gas to the mainland would free their land-produced oil for sale on the world market. So first Arco, then Occidental, Shell, BP, Exxon and many others are taking the financial plunge. Exploratory drilling contracts to Occidental, BP, and Exxon have recently been announced.

This will be one of the largest financial gambles ever made by these oil companies, and it is strongly based on an unconventional exploration technique — thermal maturation and offshore heat flow surveys. Of course, it would never have been made if the conventional seismic methods had shown anything but the largest, most seductive traps awaiting the maturation of petroleum off the southern coast of China. Wells are to be drilled beginning in early 1984, so the ultimate test of theories — the drill bit — is not far off. □

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1. *Oil and Gas Journal*, June, 1983.

Biochemistry

Dressing the SOD

from Roger H. Pain

ONE of the most intriguing points at which biology and inorganic chemistry converge — almost justifying the term 'inorganic biochemistry' — is the superoxide radical O_2^- . This negatively charged radical is a common intermediate of the reduction of oxygen and is generated by a wide range of enzymatic oxidation reactions in living, respiring cells. It is potentially damaging and indeed appears to be involved in the destruction of cells by macrophages¹ and by natural killer cells². Superoxide spontaneously undergoes a dismutation reaction

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

producing hydrogen peroxide. The reaction is fast, with a rate constant of $2 \times 10^5 M^{-1}s^{-1}$ so that O_2^- is normally present in only minute concentrations.

It is presumably an indication of the toxicity of O_2^- that an enzyme catalysing the dismutation is present in relatively high concentrations in respiring cells. This activity was only discovered in 1969 although the protein had been known for many years, masquerading under various names, including haemocuprein, hepatocuprein, erythrocuprein and cerebrocuprein. The metalloenzyme superoxide dismutase, now rejoicing in the acronym SOD, catalyses the dismutation of O_2^- with a rate constant of $2 \times 10^9 M^{-1}s^{-1}$. Taking into account the levels of enzyme and substrate in human liver, for example, this means that the decay of O_2^- in that tissue is speeded up by a factor of 10^9 (ref. 3). Just how SOD captures and catalyses the dismutation of such a small species so efficiently is beginning to become clear by looking at studies of the molecular structure of the enzyme in the light of the considerable biochemical literature on the reaction. Two important new studies of the enzyme, both from David and Jane Richardson and their colleagues at Duke University, the University of California, San Francisco and the Scripps Clinic, appear in this issue of *Nature* (p.284 and 287).

The copper, zinc superoxide dismutase comprises two identical subunits, each with an eight stranded β -barrel in the familiar Greek key conformation and three large loops of aperiodic structure. The Richardsons and their colleagues have refined the earlier 2 Å structure and included the ordered water molecules in the vicinity of the active site⁴. They find that two of the loops form the walls of a deep channel on the outside surface of the barrel, with the active site copper ion Cu(II) slightly exposed on the floor of the channel and close to a completely buried zinc ion, Zn(II). The amino acid residues making up this channel are highly con-

served in different species, suggesting that the channel is important. The active site has been identified as a small pit over the copper ion by using interactive computer graphics to fit the substrate O_2^- into position. The copper ion is held in place by four histidine residues while the neighbouring zinc is similarly held by three histidines, one of which acts as a bridge between the copper and the zinc. A feature of this region is the interlocking network of hydrogen bonds which stabilises the location of the ligands and hence the metal ions.

The formal reaction scheme for the superoxide dismutation

$$E-Cu(II) + O_2^- + H^+ \rightleftharpoons E^+-Cu(I) + O_2$$

$$E-Cu(I) + O_2 + H^+ \rightleftharpoons E-Cu(II) + H_2O_2$$

looks unexceptional. The mechanism which the authors propose, however, is both elegant and interesting. Tetrahedral distortion of the Cu(II) ligands, itself a consequence of the protein conformation, in combination with the presence of the neighbouring zinc ion, accounts for the unusually high oxidation reduction potential of the copper. The strain in the zinc-ligand conformation helps to draw the enzyme-substrate complex into the first intermediate state on the catalytic pathway. In addition, the zinc also ensures that the bridging histidine 61 picks up a hydrogen atom during the catalytic process, later to be donated to a second O_2^- to form hydrogen peroxide.

The precise location and stabilization of the active site, metal-binding residues, allowing only histidine 61 to rock gently away from the copper when the latter is in its Cu(I) state, fits one's preconceptions of an enzyme designed to catalyse the reactions of a small, rigid substrate. Other factors are required, however, to explain the particularly high rate enhancement over an already rapid reaction. The rate constant for SOD is within an order of magnitude of the theoretical limit, itself governed by the rate at which substrate can diffuse to the enzyme. This is despite the fact that the copper containing pit to which substrate must bind forms only a fraction of a percent of the total surface area exposed to the solvent. The probability of a productive interaction resulting from random collisions between SOD and O_2^- is therefore low.

In their second paper⁵ the Richardsons and their colleagues have calculated the electrostatic potential in and around the active site channel. Although an acidic protein, the long, deep channel is positively charged with residues critically placed to guide an incoming negatively charged ion towards the copper ion in the active site. It seems reasonable to suppose that the rate

enhancement arises from relatively long range electrostatic forces which talk the incoming substrate down on to the active site, so reducing the number of non-productive collisions.

SOD thus provides a particularly good example of an asymmetric distribution of charged groups on the surface of a protein which plays a role in rate enhancement. Other examples of this phenomenon are the dipolar cytochrome *c* (ref. 6) and ribonuclease T1 (ref. 7). It will be interesting to see whether a similar system operates in the iron and manganese SOD family whose structures have recently been confirmed to be completely different from that of the copper and zinc class^{8,9}.

The two new papers appearing in *Nature* are notable for the successful use of computer graphics in investigating an enzyme mechanism. The communication of structural and electrostatic field information has been tackled effectively by 'dressing' the skeleton model with electrostatic field potential and field vectors in colour, with the flair associated with the Duke University laboratory. Indeed, the illustrations on p.288 might suggest a suitable epitaph for the artistic contribution of Jane Richardson:

"She then shall dress a sweeter SOD
Than Fancy's feet have ever trod"

Carter, 1748.

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1. Roder, J.C., Kane, K. & Kiessling, R. *Progr. Allergy* **28**, 66 (1981).
2. Roder, J.C. *et al.*, *Nature* **298**, 569 (1982).
3. Fridovich, I. *Ann. Rev. Biochem.* **44**, 147 (1975).
4. Tainer, J.A., Getzoff, E.D., Richardson, J.S. & Richardson, D.C. *Nature* **306**, 284 (1983).
5. Getzoff, E.D., Tainer, J.A., Weiner, P.K., Kollman, P.A., Richardson, J.S. & Richardson, D.C. *Nature* **306**, 287 (1983).
6. Margoliash, E., Ferguson-Miller, S., Brautigan, D.L. & Chaviano, A.H. in *Structure-Function Relationships of Proteins* (eds. Markham, R. & Horne, R.W.) (North Holland, 1982).
7. Heinemann, U. & Saenger, W. *Nature* **299**, 27 (1982).
8. Ringe, E., Petsko, G.A., Yamakura, F., Suzuki, K. & Ohmori, D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3879 (1983).
9. Stallings, W.C., Powers, T.B., Patridge, K.A., Fee, J.A. & Ludwig, M.L. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3884 (1983).

100 years ago

THE following is an illustration of what private enterprise may effect for the benefit of science. When the Swedish ship *Monark* was leaving Sweden last year for Australia the second officer on board applied to the Zoological Museum at Upsala for the loan of a trawl and some vessels for preserving natural history objects. The results have been a collection of some 120 species of fish, 50 of insects and some birds.

The *Times* Calcutta Correspondent, in speaking of the possibility of opening up Thibet to Indian trade by way of Darjeeling, states that the Prime Minister of the Lama at Shigatze, said to be a most intelligent man, sent recently to Darjeeling for a supply of English books, photographic and other scientific apparatus.

From *Nature* **29**, 86; November 22, 1883.

The 1982–83 climate anomaly in the equatorial Pacific

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Disastrous climatic events, such as the Australian–Indonesian drought and the severe flooding in South America, which occurred in the equatorial Pacific in 1982/83 are part of a pattern which can be followed in unprecedented detail using satellite data. The evolution of wind, precipitation and surface temperature fields during this remarkable example of atmosphere–ocean interaction are described in this article. Models can relate the main features of the sea-surface temperature, sea level and surface current patterns to the winds, and relate wind to precipitation (atmospheric heating) patterns. The precipitation field shows a close relationship with surface temperature, but this is less well understood.

A REMARKABLE sequence of extreme climate fluctuations occurred in the tropical Pacific during 1982 and 1983. These were associated with the appearance of higher than normal sea-surface temperatures (SSTs) in the central and eastern equatorial Pacific. These anomalously warm conditions, which we will call warm episodes, are found to occur at intervals of 2–10 yr and are part of the El Niño–Southern Oscillation (ENSO) phenomenon^{1,2}. This climate fluctuation is of central importance for prediction purposes because of its global nature, strong signal, interannual time scale, and inherent lag relationships. The 1982–83 warm episode is of particular interest because of its unusual intensity and evolution. Largely because of the present system of global satellite observations, which provide information on SST, wind and precipitation fluctuations over the vast and otherwise poorly sampled ocean areas, it is by far the best observed warm episode to date.

The development of a warm episode coincides with a major change in pressure patterns which was called the Southern Oscillation³ (SO) by Walker⁴. The changes occur in a ‘seesaw’ fashion, one end of the seesaw being in the Australian–Indonesian region and the other in the south-east Pacific. A parameter which measures these seesaw changes, and so can be called a Southern Oscillation Index (SOI), is the difference in pressure between Tahiti and Darwin. Figure 1a shows 5-month running-mean values of this index, expressed as departures from normal in units of the standard deviation. The minima which appear in 1969, 1972 and 1976–77 (Darwin pressure high, Tahiti pressure low) reflect the three previous warm episodes.

In 1982, the SOI dropped precipitously between May and June and then continued to fall reaching record low values in late 1982. The associated rise in Darwin pressure reflects the filling and eastward migration of the Indonesian low-pressure trough. The eastward pressure gradient on the Equator, which is normally positive and drives the easterly trade winds, dropped to zero and then reversed in mid-1982. The consequence for the wind is illustrated in Fig. 1b, which shows the departure from the normal westward flow at 850 mbar (around 1,500 m) averaged over the area 5° N–5° S, 135° E–170° W. By July, the average flow over the index area had shifted to westerly, and remained so until December. 850 mbar, rather than the surface, is used as the reference level because of the additional wind estimates which can be derived for that level from satellite observations of low-level cloud movement.

The way that sea-surface temperature anomalies (SSTAs) developed is shown in Fig. 2 (solid line) for two representative regions of the equatorial Pacific. By June 1982, anomalies of

1°C extended over an enormous 12,000-km span from near South America to 170° E. The rapid anomaly increase in the eastern equatorial Pacific in September and October (Fig. 2 lower) approximately 3 months after the initial collapse of the easterlies in the western Pacific, marked the onset of El Niño (warm) conditions along the South American coast. The record rains and flooding during the next nine months over Ecuador and the northern provinces of Peru were part of the most catastrophic and prolonged El Niño visitation on record. The drastic biological effects have been reported by Barber and Chavez⁵.

Comparison with the six-event composite anomaly curves (broken lines in Fig. 2) of Rasmusson and Carpenter³ shows the unusual intensity and timing of the anomaly on the South American coast. Normally, the anomaly peaks in the early months of the year, well before it does in the central Pacific. In

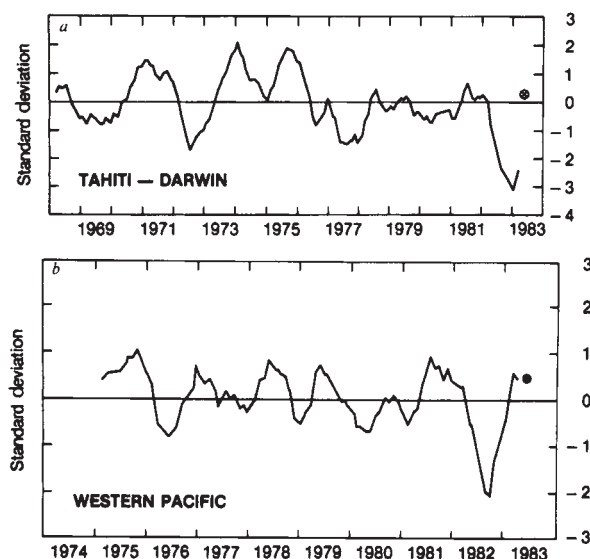


Fig. 1 Time history of *a*, the Tahiti minus Darwin atmospheric pressure anomaly at sea level, which is used as a Southern Oscillation Index (SOI). *b*, The average easterly wind speed anomaly over the western equatorial Pacific in the area between 135° E and 170° W, from 5° S to 5° N. The curves represent five month averages of normalized values, that is, the anomaly difference divided by the standard deviation for the appropriate month. The circled crosses indicate the August value.

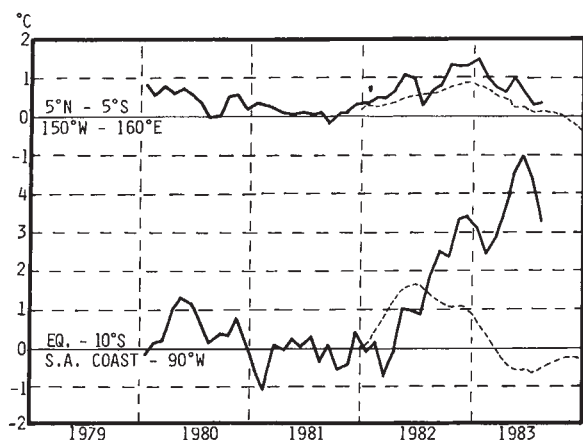


Fig. 2 SST indices. Monthly average SST anomalies ($^{\circ}\text{C}$) (solid lines) for January 1980–August 1983. Averages are for 4°S – 4°N , 160°E – 150°W (upper) and for 0° – 10°S , South American coast to 90°W (lower). The dashed curve is the composite anomaly for six warm episodes (1951, 1953, 1957, 1965, 1967, 1972).

1982, however, the warm anomalies did not appear until later in the year, and reached a first peak in December. In a further unusual turn of events, the SSTA reached a second and higher peak in June 1983 with local values in excess of 7°C . Since that time, the anomaly has been falling rapidly.

There are too many ramifications of the 1982–83 event to cover in one article, and various aspects are summarized elsewhere^{2,5–7}. Here we concentrate on various key links in the chain of processes which contributed to the anomalous behaviour of the ocean–atmosphere system during 1982–83, namely: (1) the effect of heating anomalies in the atmosphere (these are associated with precipitation anomalies) on winds; (2) the relation between heating anomalies and SST; (3) the effect of currents on temperature; and (4) the effect of the wind on ocean sea level and currents. Some simple model studies are used in conjunction with the observations to help understand some of the links.

Changes in precipitation

First we focus attention on the massive departures from the normal precipitation regime of the tropical Pacific. These changes have been monitored effectively using satellite outgoing long wave radiation (OLR) data. Regions of deep convective clouds and heavy precipitation correspond with regions of low OLR, while cloudless regions, or regions of low clouds, are regions of high OLR. Because of the relatively short satellite record, OLR anomalies are computed as departures from a 1974–78 base period.

The patterns for September 1982 and December 1982 are shown in Figs 3 and 4. Both show positive anomalies over Australia and Indonesia which experienced severe, even record, drought. However, the most prominent feature is the region of large negative anomalies (heavy precipitation) centred near the

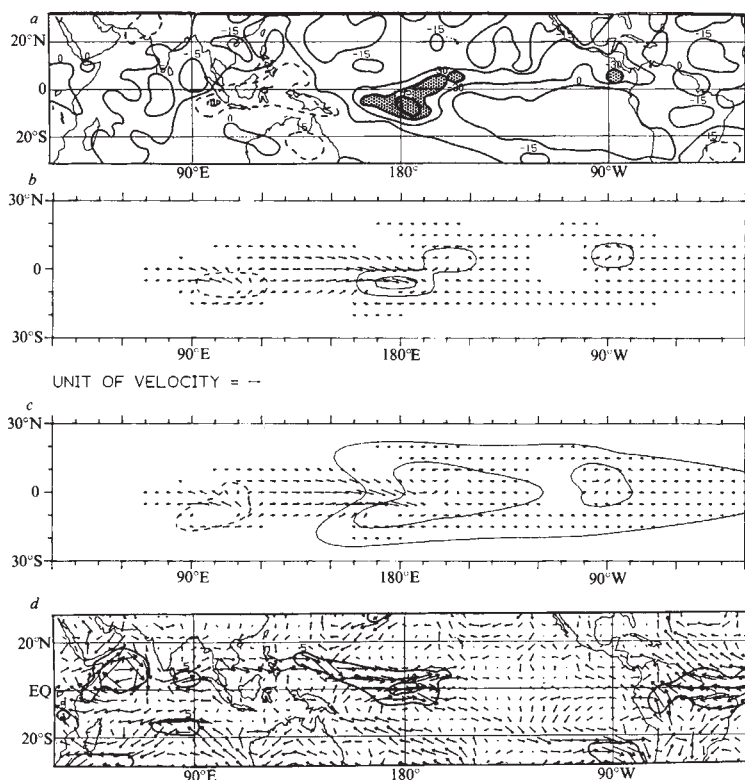


Fig. 3 *a*, Monthly mean value for September 1982 of outgoing long-wave radiation (OLR) anomaly in Wm^{-2} . Hatched regions indicate values above 30 Wm^{-2} and stippled regions values below -30 Wm^{-2} . *b*, Model response (low-level wind anomalies shown by arrows) to heat source anomalies (solid contours at 0.2 and 1.0 units), and sink anomalies (broken contour at -0.2 units) with position and strength based on *a*. *c*, As in *b*, but with surface pressure contours (interval of 0.2 units, solid contours negative, broken contour positive). *d*, Observed 850-mbar wind anomaly. Contours represent speed with 5 m s^{-1} contour interval.

date line in September and further east at $\sim 140^{\circ}\text{W}$ in December. This was associated with an eastward shift of the South Pacific Convergence Zone (SPCZ), a region of heavy precipitation which normally extends southeastwards from near New Guinea. In Kiribati (the Gilbert Islands) and the Line Islands, further east, this resulted in day after day of heavy rainfall disrupting the economy and ecology of these equatorial islands. This included the disappearance of almost the entire 17,000,000 bird population of Christmas Island⁸. Another consequence was that the tropical storm genesis region of the South Pacific shifted far east of its normal location. Hurricanes formed east of 140°W during the 1982–83 season, an unheard of event, and French Polynesia was devastated in turn by five hurricanes between December 1982 and April 1983. In contrast to the heavy rainfall of the equatorial and south-east Pacific, serious drought conditions developed along an axis from the Philippines to the Hawaiian Islands during the early months of 1983.

The monthly mean low level (850 mbar) wind anomaly patterns for September 1982 and December 1982 are also shown in Figs 3 and 4. A prominent feature which can be seen on these maps is a region of westerly anomalies located on the Equator. This first appeared in the west Pacific in June and then migrated eastwards, steadily increasing in area and magnitude. By September (Fig. 3) the anomaly was centred near the dateline, and by December (Fig. 4) it was centred at about 150°W . In fact, the large negative OLR anomaly corresponding to the heavy precipitation zone and the low-level westerly wind anomaly both migrated eastwards across the Pacific over a period of about a year (Fig. 5). This was one of the striking features of the 1982–83 warm episode, and suggests an association between the OLR anomaly and the wind anomaly.

To explore this association further, some simple model calculations were carried out. The basis for the calculations is as

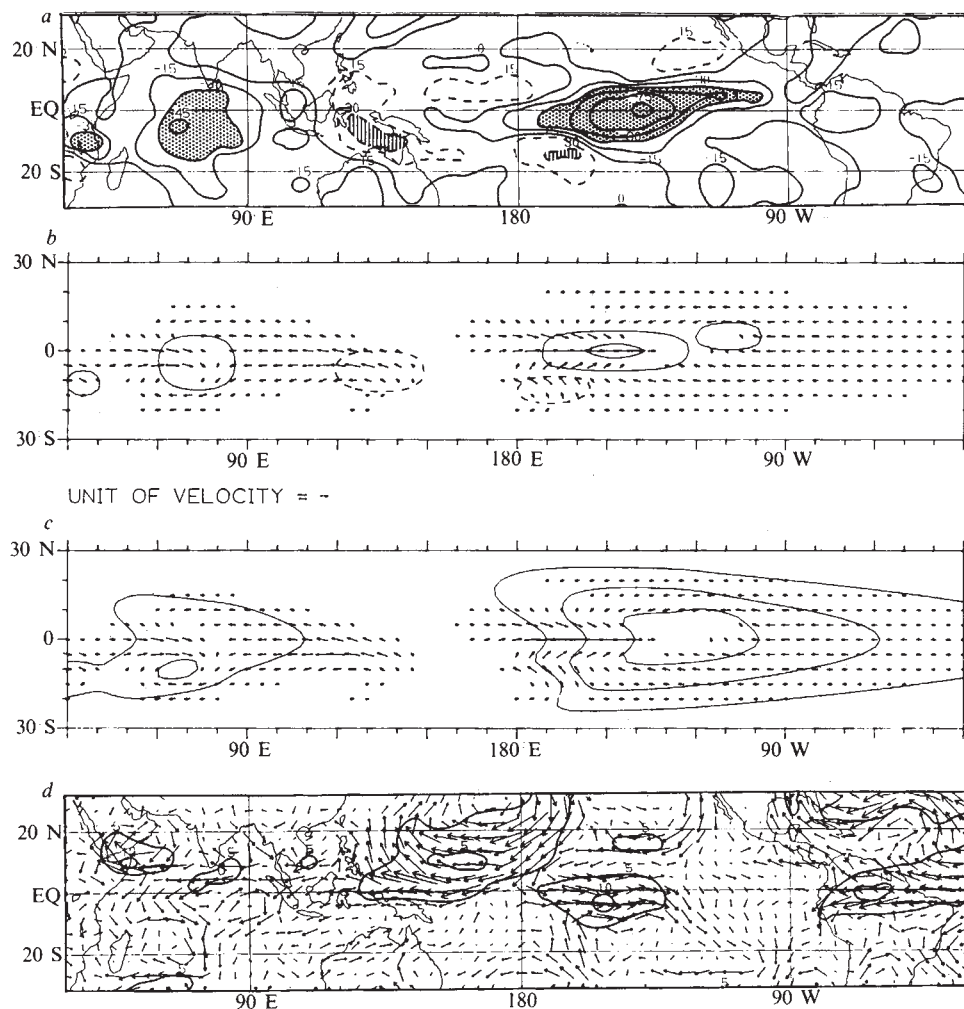


Fig. 4 Same as Fig. 3, but for December 1982. In *b*, the contours are ± 0.4 , $+2.0$ and in *c* the contour interval is 0.4 units.

follows. Cloudiness and rainfall in the tropics are associated with the release of latent heat into the atmosphere when water vapour condenses. The heating of the atmosphere tends to be a maximum at middle tropospheric levels⁹ in the tropics. Such heating drives a convection cell with low-level air flowing in at the base, being heated within the cell and flowing out again at high levels. However, the inflowing air does not come in symmetrically from all sides because of dynamical effects which force the flow to be oriented more in the east-west direction. A simple model¹⁰ which explains these asymmetries was used (with the same parameter values as in the original paper) for each month of the 1982–83 event. Elliptical¹¹ sources and sinks were used to drive the model, the location, shape and strength of these being based on the OLR anomaly maps. Only anomalies within 20° of the Equator and including contours of 30 W m^{-2} or more were included. Unit strength was given to anomalies with 30 W m^{-2} as the maximum contour, strength 1.5 was given to anomalies containing a 45 W m^{-2} contour, and so on. Example outputs showing the low-level winds produced are given for September and December 1982 in Figs 3 and 4. Within the tropical zone, the model wind anomaly field agrees remarkably well with the observed anomaly field, considering the simplicity of the model. Similar levels of agreement were found for all months. In particular, the model results show that anomalous heating located where the large OLR anomalies are found produces strong westerly anomalies in about the right location. It seems reasonable to conclude, therefore, that the observed westerly anomalies can be regarded as resulting from anomalous heating of the atmosphere. (The relationship between the heating and motion fields is in practice more complicated, for although the heating does indeed produce the winds, it depends on those very same winds to provide the moisture which fuels it.)

Convection zone

The question now is 'Why is there anomalous heating in the observed locations?' In other words, what determines the location of the convection zone which we have associated with a negative anomaly in OLR?

We will present evidence that suggests a relationship with SST, but before doing this, we will give some background on the experience obtained from general circulation models (GCMs) of the atmosphere. These models attempt to reproduce the atmospheric circulation given the radiative driving from the Sun and boundary conditions at the Earth's surface, the surface condition most relevant here being the SST. A particularly interesting calculation run at the Geophysical Fluid Dynamics Laboratory at Princeton, New Jersey, was a 15-yr simulation¹² driven by seasonally-varying radiation and seasonally-varying SST. This model showed a surprising amount of interannual variability, that is the 15 summers of the model were not all the same, even though the forcing was the same for each summer. The interannual variability did not, however, include large zonal changes in the tropics such as those found in 1982–83, so the conclusion is that these are associated with changes in the boundary conditions, that is, with changes in SST.

To follow up this idea, GCMs have been used¹³ to study the effect of SSTAs on the atmospheric circulation. The atmosphere being what it is, neither the normal SST nor the anomalous one will be in a steady state, but given sufficiently long runs or a sufficiently large ensemble of cases, systematic differences between the control and anomaly runs emerge. Many more experiments are required before the relationship between SST and rainfall patterns is understood, but some factors are emerging. One is that a 1-degree anomaly is much more effective

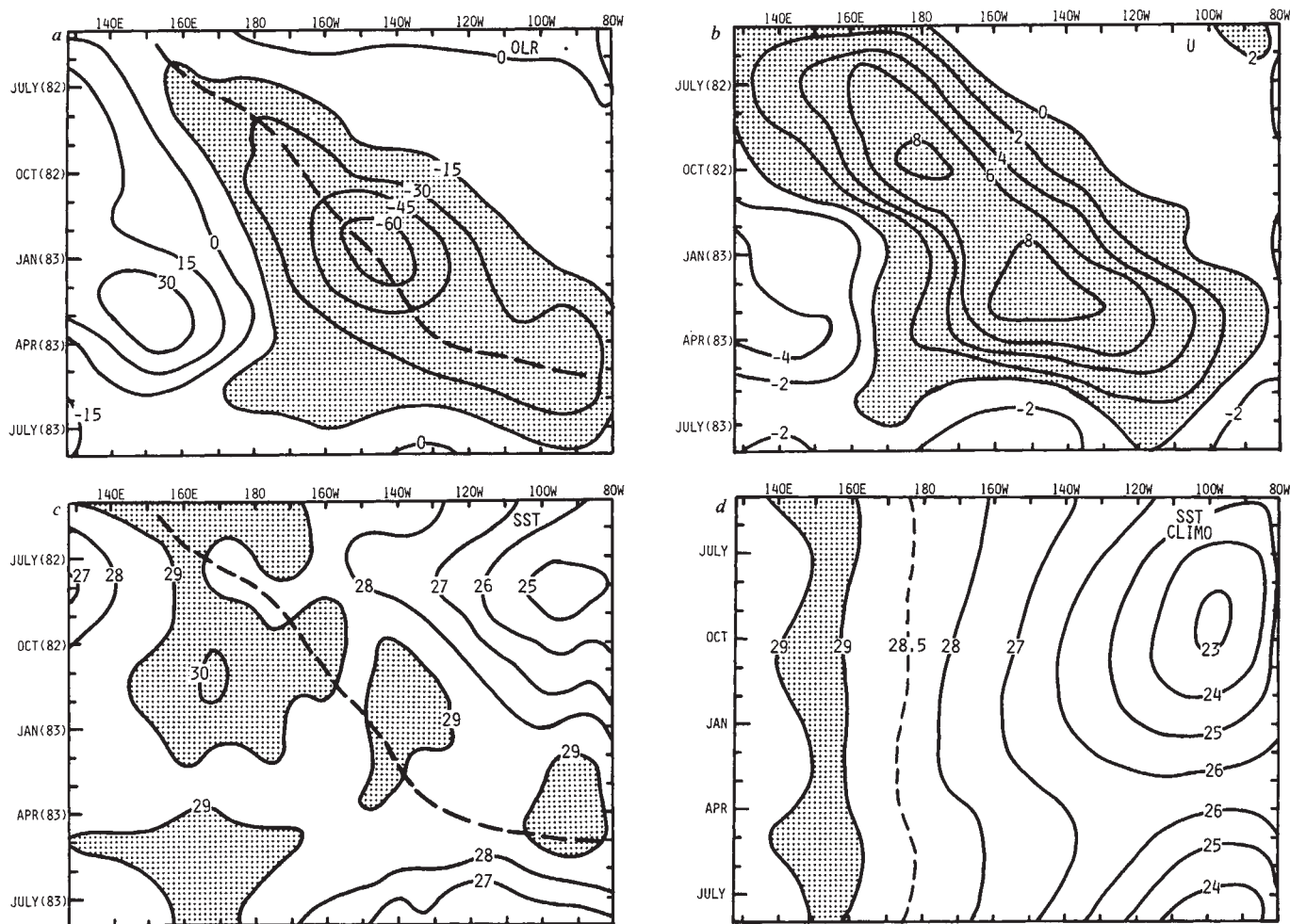


Fig. 5 Longitude-time plots along the Equator of monthly mean values of *a*, OLR anomaly during 1982–83 in Wm^{-2} . *b*, Westerly wind anomaly (5°S – 5°N) during 1982–83 in ms^{-1} . *c*, SST (4°S – 4°N) during 1982–83. *d*, SST climatological mean values (from ref. 33, slightly smoothed). The dashed line in *a*, *c* follows the peak OLR anomaly.

when the water is already warm, that is, raising the surface temperature of a certain size patch of water from 29 to 30°C will have a much bigger effect on the atmosphere than raising the temperature of a similar size patch from 19 to 20°C . (An important factor here is that the increase in the amount of moisture the atmosphere can hold is much greater in the former case.) In fact, there seems to be considerable merit in the naive idea that convection prefers to take place in the regions where the surface temperature is highest, although the relationship between SST and convection is by no means as simple as that.

A good way of presenting the observational evidence on the relationship between SST and convection for the 1982–3 event is by means of longitude-time plots of these quantities along the Equator. The OLR anomaly is a good guide to the position of the anomalous region of convection, and Fig. 5*a* shows this migrated slowly eastward crossing the entire Pacific Ocean in the 16-month period depicted. The strength of the anomaly built up steadily in the first 8 months, then fell from its peak value of 68 Wm^{-2} in January to $<15 \text{ Wm}^{-2}$ by August 1983. As expected from the atmospheric model, the wind anomaly (Fig. 5*b*) followed the OLR anomaly across the ocean. However, the SST pattern (Fig. 5*c*) also showed a migration, as seen by following water over 29°C between May 1982 and May 1983. This is in marked contrast to the normal seasonal progression shown in Fig. 5*d*. One way of describing the change is to refer to the water over 29°C as the 'warm pool' which is normally located in the western Pacific¹⁴. In 1982–1983, this warm pool migrated eastwards and the zone of anomalous convection followed it. (Note that it is the SST field that the convection zone followed. The SSTA field developed quite differently.)

Warm pool migration

The next obvious question is why did the warm pool migrate? Some useful evidence about this comes from a study¹⁵ of the 1972 warm event when the warm pool also migrated. The changes in temperature in the central Pacific in 1972 did not match those expected from heat exchange with the atmosphere, but they did match those estimated from the effect of anomalous advection, even though the advection was estimated indirectly from the sea-level data in the east Pacific. The picture that emerged from 1972 was one of eastward migration of the warm pool due to anomalous advection followed by large heat losses to the atmosphere from the abnormally warm water. The suggestion is that a similar scenario is applicable to the 1982–83 event.

So what causes the anomalous currents? There is little doubt that the major cause is anomalies in the surface winds^{16–22}, particularly through the zonal component of the wind stress anomaly in the near-equatorial band. In fact, models have been quite successful in hindcasting sea level changes due to wind stress changes which are very often at some remote location. The information about the wind changes is carried along the equatorial waveguide²³ by an eastward propagating Kelvin wave (wave speed 60° per month) and by westward propagating planetary waves (wave speeds up to 20° per month).

The results of a simple model¹⁵ calculation designed to show the principle features of the ocean response for the 1982–83 event are shown in Fig. 6*b, c*. The forcing wind stress anomaly is assumed to vary with longitude and time as shown in Fig. 6*a* (see Fig. 5*b*), and to vary with latitude θ according to the formula $(3 - \theta_x^2) \exp(-\frac{1}{4}\theta_x^2)$ where $\theta_x = \theta/5.8^{\circ}$ (zero crossing

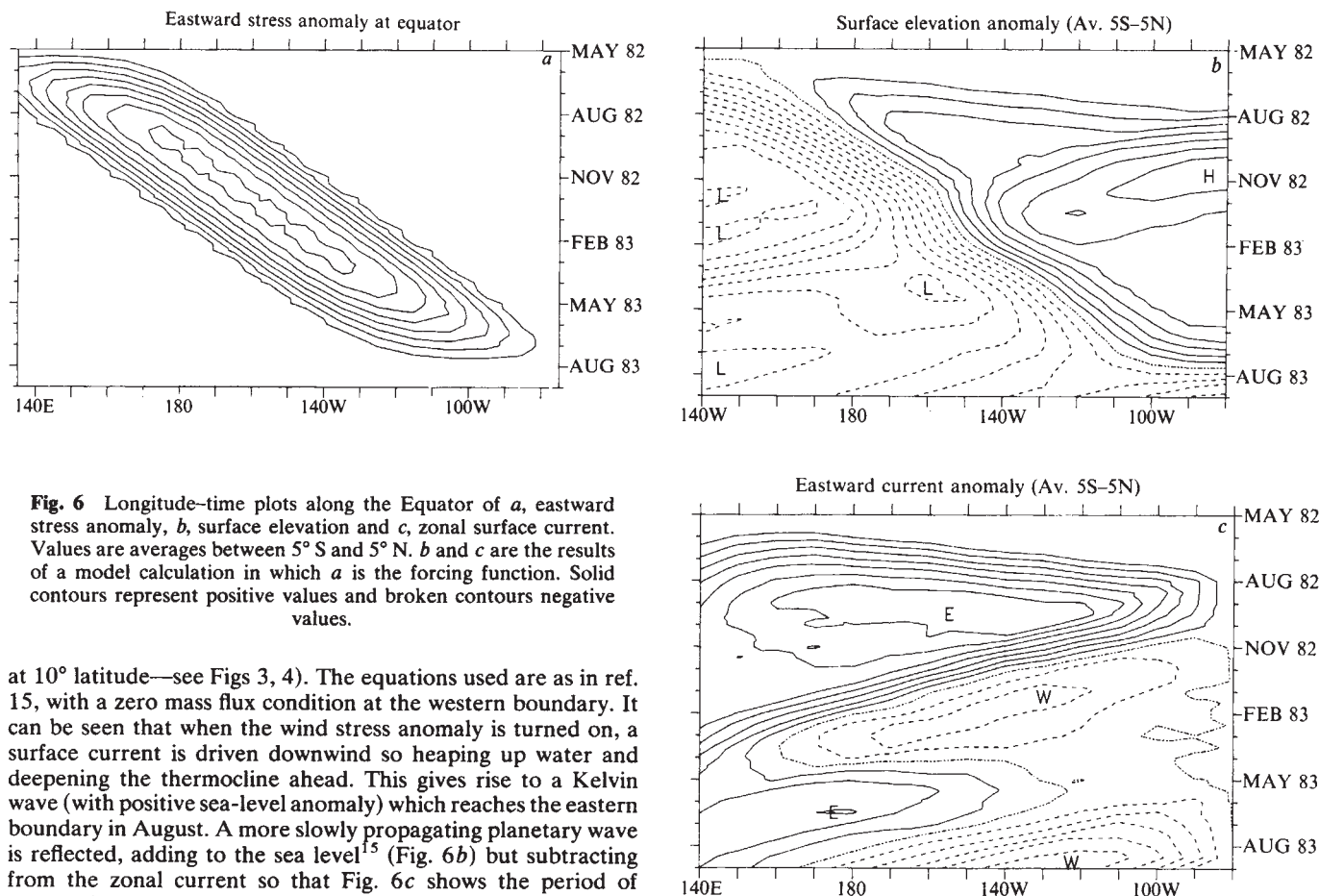


Fig. 6 Longitude-time plots along the Equator of *a*, eastward stress anomaly, *b*, surface elevation and *c*, zonal surface current. Values are averages between 5° S and 5° N. *b* and *c* are the results of a model calculation in which *a* is the forcing function. Solid contours represent positive values and broken contours negative values.

at 10° latitude—see Figs 3, 4). The equations used are as in ref. 15, with a zero mass flux condition at the western boundary. It can be seen that when the wind stress anomaly is turned on, a surface current is driven downwind so heaping up water and deepening the thermocline ahead. This gives rise to a Kelvin wave (with positive sea-level anomaly) which reaches the eastern boundary in August. A more slowly propagating planetary wave is reflected, adding to the sea level¹⁵ (Fig. 6*b*) but subtracting from the zonal current so that Fig. 6*c* shows the period of eastward anomaly ending when the planetary wave goes by. There is some 'sloshing' on a time scale of 9 months—the minimum time for a signal to cross the ocean both ways.

The agreement with sea-level observations of Wyrki (reported by Cane⁷) is remarkable, considering the simplicity of the model. For instance, at Rabaul (4° S, 152° E) the level fell 20 cm between May and December 1982, then started to rise slowly. At Fanning (4° N 159° W) Island, sea level rose about 25 cm from June to September, then fell a similar amount from September to January. At Santa Cruz (1° S 90° W) sea level rose 30 cm from August to December/January but was lower in February and March. What data there are on equatorial surface currents^{24–27} indicate strong eastward anomalies in the east and central Pacific from August to December, with reversed flow from January to March. Figure 6*c* shows a similar pattern, but with the reversal a little early.

Conclusions

The above calculations show that, for a strong event such as 1982–83, the more prominent anomalies in both atmosphere and ocean can be modelled. The calculations do not show how the event was started, but suggest reasons for the strength and persistence of an event once it has started. For it seems that once a region of anomalous convection in the atmosphere is established over the equatorial Pacific, there is a feedback process which can strengthen it. The process is that the westerly wind anomaly created to the west of the convection zone tends to drive a current downwind. This shifts the warm pool downwind, so causing the convection region to migrate eastwards. The further it moves, the greater the anomaly because the contrast between the warm pool temperature and the normal temperature gets larger with distance eastwards. Note, however, that the eastward current anomaly remains strong only for a limited time (of the order of 9 months) because of wave reflections and the finite size of the basin. Thus the central Pacific anomalies reach their peak when the current anomaly reverses sign at the beginning of 1983.

The above discussion relates mainly to the temperature distribution in the west and central Pacific. In the east, there is another important feature, namely strong seasonal variations associated with a 'cold tongue' of surface water (bounded by the 26° isotherm, say). This appears near the coast around April, then pokes out westward to reach its maximum extent in September, and has dissolved by the following March. Two important processes controlling the tongue^{28,29} are upwelling of cold water along the South American coast and advection off shore. During warm events, the tongue is much less cold than normal. One contributory factor¹⁵ is that the thermocline on the eastern boundary is much thicker³⁰ during warm events, so the source region for upwelling is much warmer, and the 1982–83 event is no exception³¹. As the thickness of the thermocline is related to sea level³⁰, the model result of Fig. 6*b* would suggest that upwelled water would be much warmer than usual from October 1982 to June 1983, and above normal for the whole period of positive surface elevation. Another factor is the coastal currents which are more strongly poleward in warm events, including³² 1982–83. The model¹⁵ suggests the maximum effect would be when the sea level is highest, although in practice a lag would be expected. Thus the model results of Fig. 6*b* relate quite well to the observed temperature anomalies in the east.

Looking ahead, the model result of Fig. 6*b* would indicate that the conditions for maintaining the anomaly have gone, and actually reversed. In addition, study of the 1972 warm episode¹⁵ suggests that there is a large anomaly in heat loss from ocean to atmosphere when the ocean is abnormally warm, so when the warm water returns to its normal position, its volume will be reduced and the warm pool may need to build up again to provide favourable conditions for another warm episode.

The 1982–83 warm event was certainly a very strong one, and seems likely to go down in history as the warm event of the century. We are fortunate in having the satellite information which allows us to describe and follow the event in unprecen-

ted detail with, for example, maps of the atmospheric winds and of radiation anomalies. Despite this increased knowledge of the atmospheric changes, there is still a great deal that is unknown and unanswered, particularly in the ocean. Clearly it would be beneficial to be able to model the progress of warm events in the same way as we currently model the development of weather systems, but this will require greatly expanded observing networks in the tropical oceans.

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1. Philander, S. G. H. *Nature* **302**, 295–301 (1983).
2. Philander, S. G. H. *Nature* **305**, 16 (1983).
3. Rasmusson, E. M. & Carpenter, T. H. *Mon. Weath. Rev.* **110**, 354–384 (1982).
4. Walker, G. T. *Mem. Ind. met. Dept* **24**, 275–332 (1924).
5. Barber, R. T. & Chavez, F. *Science* (in the press).
6. Rasmusson, E. M. & Wallace, J. M. *Science* (in the press).
7. Cane, M. *Science* (in the press).
8. Shreiber, R. W. & Schreiber, E. A. *Trop. Ocean-Atmos. Newslett.* **16**, 10–12 (1983).
9. Newell, R. E., Kidson, J. W., Vincent, D. G. & Boer, G. J. *The General Circulation of the Tropical Atmosphere* Vol 2 (MIT Press, Cambridge, 1974).
10. Gill, A. E. *Q. Jl R. met. Soc.* **106**, 447–462 (1980).
11. Heckley, W. A. & Gill, A. E. *Q. Jl R. met. Soc.* **40**, 1613–1630 (1983).
12. Lau N.-C. *Mon. Weath. Rev.* **109**, 2287–2311 (1981).
13. Shukla, J. & Wallace, J. M. *J. atmos. Sci.* **40**, 1613–1630 (1983).
14. Niiler, P. & Stevenson, J. *J. mar. Res. Suppl.* **40**, 465–480 (1982).
15. Gill, A. E. *J. phys. Oceanogr.* **13**, 586–606 (1982).
16. Gill, A. E. Models of equatorial currents. In *Numerical Models of Ocean Circulation*, 181–203 (National Academy of Science, Washington DC, 1975).
17. Hickey, B. J. *J. phys. Oceanogr.* **5**, 460–475 (1975).
18. Wyrtki, K. *J. phys. Oceanogr.* **5**, 572–584 (1975).
19. Barnett, T. P. *J. phys. Oceanogr.* **11**, 1043–1058 (1981).
20. McCreary, J. J. *J. phys. Oceanogr.* **6**, 632–645 (1976).
21. Busalacchi, A. J. & O'Brien, J. J. *J. geophys. Res.* **86**, 10901–10907 (1981).
22. Busalacchi, A. J., Takeuchi, K. & O'Brien, J. J. in *Hydrodynamics of the Equatorial Ocean* (ed. Nihoul, J. C. J.) 155–195 (Elsevier, Amsterdam, 1983).
23. Gill, A. E. *Atmosphere-Ocean Dynamics* (Academic, New York, 1982).
24. Lukas, R. & Firing, E. *Trop. Ocean-Atmos. Newslett.* **16**, 9–10; **21**, 9–11 (1983).
25. Vitousek, M. *Trop. Ocean-Atmos. Newslett.* **16**, 10 (1983).
26. Toole, J. M. *Trop. Ocean-Atmos. Newslett.* **16**, 12–14 (1983).
27. Halpern, D., Hayes, S. P., Leetmaa, A., Hansen, D. V. & Philander, S. G. H. *Science* (in the press).
28. Leetmaa, A. *J. phys. Oceanogr.* **13**, 467–473 (1983).
29. Philander, S. G. H. & Pacanowski, R. C. *Tellus* **33**, 201–210 (1981).
30. Gill, A. E. *J. phys. Oceanogr.* **12**, 1372–1387 (1982).
31. Smith, R. L. & Huyer, A. *Trop. Ocean-Atmos. Newslett.* **21**, 28–29 (1983).
32. Smith, R. L. *Science* (in the press).
33. Reynolds, R. W. A *Monthly Averaged Climatology of Sea Surface Temperature*, NOAA Tech. Rep. NWS **31** (1982).

A polymorphic DNA marker genetically linked to Huntington's disease

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|| Venezuela Collaborative Huntington's Disease Project*

Family studies show that the Huntington's disease gene is linked to a polymorphic DNA marker that maps to human chromosome 4. The chromosomal localization of the Huntington's disease gene is the first step in using recombinant DNA technology to identify the primary genetic defect in this disorder.

HUNTINGTON'S disease (also known as Huntington's chorea) is a progressive neurodegenerative disorder with autosomal dominant inheritance¹. The first symptoms of Huntington's disease usually occur in the third to fifth decade and the gene is completely penetrant. The disease is characterized by both progressive motor abnormality, typically chorea, and intellectual deterioration commonly accompanied by prominent psychiatric symptoms including severe depression. The symptoms of Huntington's disease result from premature neuronal cell death which is most marked in the basal ganglia. Although the prevalence of Huntington's disease is only 5–10 in 100,000, because of its late onset most individuals have children before they realize they have inherited Huntington's disease, and it thus significantly affects a much greater proportion of the population². In spite of numerous biochemical studies of peripheral tissues and of post-mortem brain tissue the primary defect in Huntington's disease has never been detected^{3,4}. At present there is no reliable method of presymptomatic or prenatal diagnosis of the disease and there is no effective therapy.

A number of investigators have examined Huntington's disease pedigrees in search of a genetic marker linked to the

Huntington's disease locus^{5,6}. Such studies have proved difficult because of the late age of onset of the disorder and the consequent need for a large number of individuals to be typed. Although investigations relying on classical polymorphic antigen and enzyme markers were uniformly negative, they did exclude the Huntington's disease locus from 20% of the human genome⁵. The lack of additional polymorphic protein markers precluded the possibility of testing remaining regions of the genome.

In recent years, the techniques for circumventing this difficulty have been developed^{7,8}. Recombinant DNA technology provides a method for obtaining the requisite number of new genetic markers because it permits the monitoring of heritable differences in the sequence of genomic DNA. These DNA markers are termed restriction fragment length polymorphisms (RFLPs). They are detected as differences in the sizes of restriction fragments observed in Southern blotting experiments on human genomic DNA using cloned DNA probes free of repetitive sequences. Unlike classical expressed markers, DNA polymorphisms can be found in regions of the genome irrespective of whether they encode a protein. Probes that detect RFLPs can therefore be derived from known gene loci or from anonymous DNA segments. RFLPs appear to be present in all regions of the genome, thus making it feasible to construct a detailed human genetic linkage map and thereby localize disease genes^{7–11}. We have now identified an anonymous DNA fragment from human chromosome 4 that detects two different RFLPs in a *HindIII* digest of human genomic DNA. This polymorphic DNA marker shows close genetic linkage to the Huntington's

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disease gene in two separate families, although a different haplotype of the marker segregates with the Huntington's disease gene in each family. We infer that the Huntington's disease locus resides on human chromosome 4.

Huntington's disease families

The success of any genetic linkage project depends in large part on the quality of family material available for study. We initially invested considerable effort in identifying a useful family from the National Research Roster for Huntington's Disease Patients and Families at Indiana University. An American family of reasonable size was selected (Fig. 1) and blood samples were obtained to establish permanent Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines. Subsequently, a substantially larger Huntington's disease family was located (Fig. 2)¹². This pedigree stems from a unique community of interrelated Huntington's disease gene carriers living along the shores of Lake Maracaibo, Venezuela. In a pedigree numbering over 3,000 since the early 1800s, all Huntington's disease patients have inherited the defective gene from a common ancestor. This is the largest known concentration of Huntington's disease in one family. For the past three years, an expedition has spent one month annually in Venezuela collecting pedigree information, tissue samples, and clinical data (N. S. Wexler *et al.*, manuscript in preparation). Permanent lymphoblastoid cell lines were again established for each individual to act as a permanent source of genomic DNA. In both families, each individual was examined by at least one neurologist experienced and knowledgeable about Huntington's disease. In Venezuela, many family members were examined for three consecutive years. Individuals were diagnosed as having Huntington's disease on the basis of family history, abnormal motor function and intellectual impairment. Each was assigned to a functional stage reflecting physical and social functioning according to a scale developed by Shoulson and Fahn¹³. Blood samples from members of both pedigrees were analysed using at least 20 red cell and plasma markers to exclude cases of non-paternity.

Characterization of bacteriophage clone G8

A number of DNA probes that detect RFLPs have been identified in this and other laboratories. Some of these probes represent DNA sequences from known gene loci whilst others are anonymous DNA segments chosen because they contain no repetitive DNA sequences. In an initial screen for linkage to the Huntington's disease gene, 12 such DNA markers were tested in the American family depicted in Fig. 1. Of these markers only one, G8, gave a suggestion of linkage to the Huntington's disease gene¹⁴.

The G8 clone is a recombinant bacteriophage from the human gene library of Maniatis and co-workers¹⁵. It contains 17.6 kilobases (kb) of human DNA free of repetitive sequences. In our previous studies, G8 DNA was used as a probe in Southern blotting experiments in an attempt to identify RFLPs¹⁶. The probe detected two invariant and several variable *Hind*III fragments in human genomic DNA. The pattern of hybridization of G8 to *Hind*III-digested DNAs from members of two families is shown in Fig. 3. The invariant *Hind*III fragments are seen at 8.4 kb and 2.3 kb. Other fragments whose presence varied from individual to individual (in these and other unrelated individuals not shown in the figure) are seen at 17.5 kb, 15 kb, 4.9 kb, 3.7 kb and 1.2 kb. In order to determine the basis for this variation, we constructed a restriction map of clone G8.

In our initial attempts to derive this map, we determined that the G8 insert contained five *Eco*RI fragments of 6.0 kb, 5.5 kb, 2.4 kb, 2.2 kb and 1.5 kb. We subcloned each of these *Eco*RI fragments into a plasmid vector (pBR322 or pUC9). *Hind*III sites within each *Eco*RI fragment were mapped by direct digestion of the subcloned DNA with *Hind*III. The order of the *Eco*RI fragments within the bacteriophage clone was determined by using each subclone as a probe against single or double *Hind*III and *Eco*RI digests of G8 DNA.

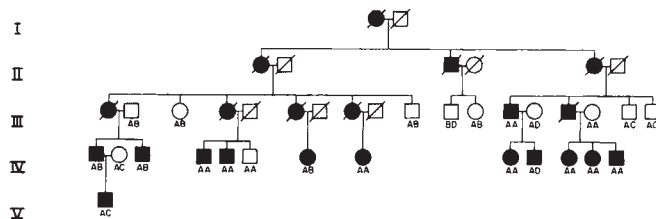


Fig. 1 Pedigree of an American Huntington's disease family. Symbols: circles, females; squares, males; a black symbol indicates that an individual is affected with Huntington's disease; a slashed symbol indicates that an individual is deceased. This pedigree was identified through the National Research Roster for Huntington's Disease Patients and Families at Indiana University. Relevant family members were examined by a neurologist and blood samples were obtained. EBV-transformed lymphoblastoid cell lines were established for the individuals whose genotypes are shown and have been stored at the Human Genetic Mutant Cell Repository, Camden, New Jersey. Phenotypes at the G8 locus shown under each symbol were determined by Southern blotting as outlined in Fig. 3. For the purposes of confidentiality, selected individuals are not shown.

Variation at one *Hind*III site, called site 1, occurs outside of the actual cloned sequence in G8. It is detected in Southern blot experiments by hybridization to sequences at the left end of the map shown in Fig. 4. When this *Hind*III site is present, a 15 kb fragment is seen; when it is absent a 17.5 kb fragment is seen. The second *Hind*III RFLP results from the presence or absence of a site, called site 2, within the G8 insert. When this site is present, two fragments are seen, 3.7 kb and 1.2 kb, but in its absence, only a single fragment of 4.9 kb is detected.

A survey of 23 unrelated North Americans gave allele frequencies for *Hind*III site 1, occurring outside the clone, of 0.74 for the absence of the site, and 0.26 for the presence of the site. For *Hind*III site 2, mapping within the G8 clone, allele frequencies were 0.81 for the presence of the site and 0.19 for the absence of the site. In each case we have termed the more frequent allele 1, and the minor allele 2 (see Fig. 3). Alterations in restriction fragment length due to the presence or absence of each of these sites display a Mendelian pattern of inheritance. Each *Eco*RI subclone was also used as a probe against *Hind*III digests of genomic DNA. The restriction map of the insert in G8 and the corresponding genomic region including the positions of the two variable *Hind*III sites is shown in Fig. 4.

When polymorphic sites in the DNA map are in such close proximity that the frequency of recombination between them is negligible, they are inherited together as a unit. The combined information from both sites on a given chromosome can then be considered as a single haplotype. At the G8 locus, the two polymorphic *Hind*III sites are separated by only 18.7 kb. Family studies were carried out using members of the Venezuela pedigree heterozygous at both sites to test for co-segregation of the two RFLPs. Because of the predictive nature of the data, the phenotypes observed for these 'at risk' individuals are not shown to protect their confidentiality. The data were analysed using the computer program LIPED¹⁷. A lod score of 4.53 was obtained at a recombination fraction (θ) of 0.0. No crossovers were seen between the two sites, supporting the hypothesis that the RFLPs are inherited together as a haplotype. The four possible combinations of alleles at site 1 and site 2 have been termed haplotypes A, B, C and D as explained in Fig. 4.

If no linkage disequilibrium exists between the two polymorphic *Hind*III sites then the frequency of these haplotypes in the population will be 0.61, 0.14, 0.20, and 0.05 respectively. These frequencies are predicted from the allele frequencies at each polymorphic *Hind*III site. In our limited sample of 23 individuals, we observed frequencies for A, B, C and D of 0.64, 0.11, 0.22 and 0.04 indicating that there is not a high degree of disequilibrium in the general North American population between these two RFLPs. A much larger sample of unrelated individuals will be necessary to determine with certainty whether

Fig. 2 Pedigree of the Venezuelan Huntington's disease family. This pedigree represents a small part of a much larger pedigree that will be described in detail elsewhere. Permanent EBV-transformed lymphoblastoid cell lines were established from blood samples of these individuals (unpublished data). DNA prepared from the lymphoblastoid lines will be used to determine the phenotype of each individual at the G8 locus as described in Fig. 3. The data were analysed for linkage to the Huntington's disease gene using the program LIPED¹⁷ with a correction for the late age of onset⁵. Because of the high frequency of the Huntington's disease gene in this population some of the spouses of affected individuals have also descended from identified Huntington's disease gene carriers. In none of these cases, however, was the unaffected individual at significantly greater risk for Huntington's disease than a member of the general population. Although a number of younger at-risk individuals were also analysed as part of this study, for the sake of these family members the data are not shown due to their predictive nature. The data are available upon request if confidentiality can be assured.

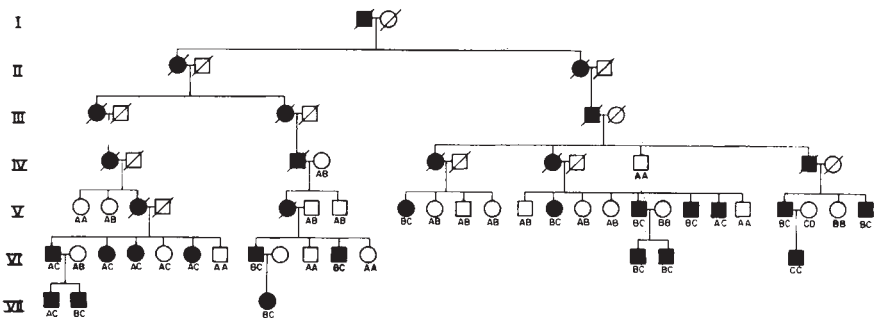
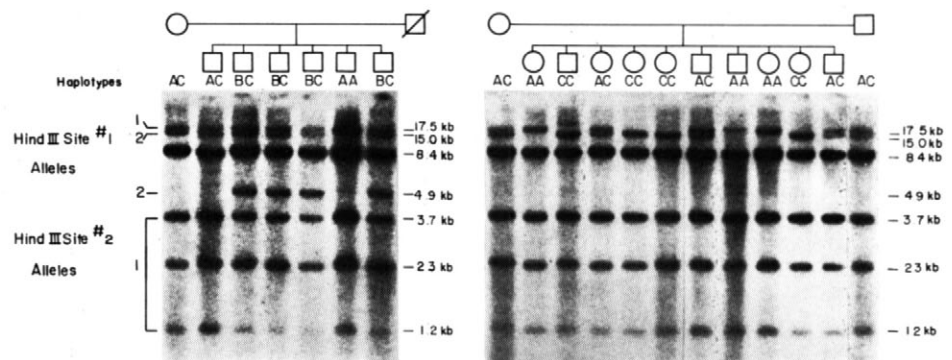


Fig. 3 Hybridization of the G8 Probe to *Hind*III-digested human genomic DNA.

Methods: DNA was prepared as described²³ from lymphoblastoid cell lines derived from members of two nuclear families. 5 µg of each DNA was digested to completion with 20 units of *Hind*III in a volume of 30 µl using the buffer recommended by the supplier. The DNAs were fractionated on a 1% horizontal agarose gel in TBE buffer (89 mM Tris, pH 8, 89 mM Na borate, 2 mM Na EDTA) for 18 h. *Hind*III-digested AC1857 DNA was loaded in a separate lane as a size marker.

The gels were stained with ethidium bromide (0.5 µg ml⁻¹) for 30 min and the DNA was visualized with UV light. The gels were incubated for 45 min in 1 M NaOH with gentle shaking and for two successive 20 min periods in 1 M Tris, pH 7.6, 1.5 M NaCl. DNA from the gel was transferred in 20×SSC (3 M NaCl, 0.3 M Na citrate) by capillary action to a positively charged nylon membrane. After overnight transfer, agarose clinging to the filters was removed by washing in 3×SSC and the filters were air dried and baked for 2 h under vacuum at 80 °C. Baked filters were prehybridized in 500 ml 6×SSC, 1×Denhardt's solution (0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidone, 0.02% Ficoll), 0.3% SDS and 100 µg ml⁻¹ denatured salmon sperm DNA at 65 °C for 18 h. Prehybridized filters were washed extensively at room temperature in 3×SSC until no evidence of SDS remained. Excess liquid was removed from the filters by blotting on Whatman 3MM paper and damp filters were placed individually in heat-sealable plastic bags. 5 ml of hybridization solution (6×SSC, 1×Denhardt's solution, 0.1% SDS, 100 µg ml⁻¹ denatured salmon sperm DNA) containing approximately 5×10⁶ c.p.m. of nick-translated G8 DNA (specific activity ~2×10⁸ c.p.m. µg⁻¹)²⁴ was added to each bag which was then sealed and placed at 65 °C for 24–48 h. Filters were removed from the bags and washed at 65 °C for 30 min each in 3×SSC, 2×SSC, 1×SSC and 0.3×SSC. The filters were dried and exposed to X-ray film (Kodak XR-5) at -70 °C with a Dupont Cronex intensifying screen for 1 to 4 days. The haplotypes observed in each individual were determined from the alleles seen for each *Hind*III RFLP (site 1 and 2) as explained in Fig. 4.



any significant degree of disequilibrium exists. The predicted level of heterozygosity at the G8 locus if the two sites are in equilibrium is 57%, making this an excellent genetic marker.

Mapping of G8 to chromosome 4

The G8 sequence was mapped to a human chromosome by Southern blot analysis of human-mouse somatic cell hybrids¹⁸. The presence or absence of fragments detected by the G8 probe was determined for 18 karyotyped hybrids (Table 1). These fragments were always seen when chromosome 4 was present in the hybrid cell line, but never seen when chromosome 4 was absent. For all other chromosomes there were several discordant clones. Seven of the hybrids were tested using the enzyme *Hind*III, whilst the rest were monitored after *Eco*RI digestion. In both cases, all fragments detected in total genomic DNA co-segregated with chromosome 4.

An additional 28 hybrids were tested only for enzyme markers previously assigned to each of the human chromosomes. Two discordant clones were seen with the chromosome 4 marker peptidase S, probably due to the relative insensitivity of the peptidase S assay compared to Southern blot hybridization. A much higher rate of discordance (>26%) was seen for all other human chromosomes. These data clearly assign the fragments detected by G8 to chromosome 4. The G8 DNA segment was recently given the designation D4S10 at The Human Gene Mapping Workshop 7¹⁹.

Analysis of linkage

After initial data were obtained for American Huntington's disease family, selected members of the Venezuela family were typed using marker G8. The haplotypes observed in these families are displayed in Figs 1 and 2. The data were analysed for linkage of the DNA marker to the Huntington's disease gene using the program LIPED¹⁷. At-risk individuals included in the analysis were assigned a probability of having inherited the gene based on an age of onset function as previously described.⁵ The cumulative distribution of age of onset was assumed to be similar in the American and Venezuelan families.⁵ Haplotype frequencies used for the G8 marker were those predicted from the individual allele frequencies for the site 1 and site 2 RFLPs assuming complete equilibrium. It should be noted that these frequencies were observed in the North American population and their exact values in the Venezuela population might vary from these estimates. Results of the analysis for G8 and Huntington's disease, together with those for two other chromosome 4 markers are given in Table 2.

The maximum lod score for Huntington's disease against G8 was 8.53 at a θ of 0.00, suggesting that the two loci are very closely linked. The maximum lod score was obtained at a θ of 0.00 for both sexes. No recombinants can be detected in either pedigree. The Huntington's disease gene segregated with the A haplotype of G8 in the American family and with the C in the Venezuela pedigree.

Table 1 Segregation of G-8 with human chromosomes in human-mouse hybrids

Cell hybrid	Chromosomes																						Translocated chromosomes
	G-8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
WIL-5	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+
WIL-2	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	+	+
WIL-7	-	-	+	+	-	+	+	-	+	-	+	+	-	+	+	-	-	+	+	-	-	+	+
JSR-22H	+	-	-	-	+	-	+	-	-	-	+	+	-	-	+	-	-	+	+	-	+	+	-
XTR-22	+	-	+	-	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+	+	+	+	-
TSL-2	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	+	-	+	+	+
JSR-17S	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
ATR-13	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-
WIL-6	+	-	-	-	+	+	+	+	+	-	+	+	-	-	+	-	-	+	-	+	+	+	+
NSL-5	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	-	+	-	-
NSL-9	-	-	-	-	-	+	-	-	+	-	+	-	+	+	+	+	+	+	-	-	+	+	-
NSL-15	+	-	+	-	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	-	-	+	+
NSL-16	+	-	-	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
REW-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+
REW-7	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
REW-10	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
REW-11	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+
JWR-26C	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+
% Discordancy*	38	31	38	7†	35	37	30	32	38	46	32	54	46	43	48	50	57	26	50	50	44	36	32
28 hybrids																							

DNA was isolated²² from an aliquot of cells at the same passage that karyotypic and enzyme analyses were performed. The presence of G8 sequences was determined by hybridization of the probe to filters containing *Hind*III-digested (first seven hybrids) or *Eco*RI-digested (remaining hybrids) cell hybrid DNA. Under the hybridization conditions used²² G8 did not detect hybridizing fragments in mouse DNA. A hybrid cell was considered positive for a given human chromosome only if it was present in greater than 10% of the cells. Some hybrids were made from fibroblasts containing the following translocation chromosomes: ATR-5/X, (5pter→5q35:Xq22→Xqter); JSR-7q⁻, (7pter→7q22); NSL-17/9, (17qter→17p11::9q12→9qter); 9/19, (9pter→9q12::17p11→17pter); JSR-2/1, (2pter→2q37::1p21→1pter); 1p⁻, (1qter→1p21::2q37→2pter); TSL-3/17, (3qter→3p21::17p13→17pter); XTR-X/3, (Xpter→Xq28::3q21→3qter).

* Twenty-eight additional hybrid cells were analysed for their chromosome content using only marker enzymes previously assigned to each human chromosome. The references for the gene assignments and assay methods are given in ref. 18. The markers tested are: chromosome 1, adenylate kinase-2, peptidase-C; 2, malate dehydrogenase (soluble), isocitrate dehydrogenase (soluble); 3, aminoacylase-1, DNA segment (D3S1); 4, peptidase S; 5, hexosaminidase B; 6, malic enzyme, superoxide dismutase (mitochondrial); 7, phosphoserine phosphatase, beta-glucuronidase; 8, glutathione reductase; 9, adenylate kinase-1, aconitase (soluble); 10, glutamate-oxaloacetate transaminase; 11, esterase-A4, lactate dehydrogenase-A; 12, lactate dehydrogenase-B, peptidase-B; 13, esterase-D; 14, nucleoside phosphorylase; 15, mannose phosphate isomerase, pyruvate kinase (muscle form); 16, adenine phosphoribosyl transferase; 17, galactokinase; 18, peptidase-A; 19, glucose phosphate isomerase, peptidase-D; 20, adenosine deaminase; 21, superoxide dismutase (soluble); 22, aconitase (mitochondria), DNA segment (D22S1); X, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase.

† The two discordant clones were positive for G8 and negative for peptidase-S. These discordancies are probably due to the greater sensitivity of Southern blot hybridization compared to the peptidase-S stain.

Table 2 lod scores

	Recombination fraction (θ)					
	0.0	0.05	0.1	0.2	0.3	0.4
Huntington's disease against G8	A 1.81	1.59	1.36	0.90	0.48	0.16
	V 6.72	5.96	5.16	3.46	1.71	0.33
	T 8.53	7.55	6.52	4.36	2.19	0.49
Huntington's disease against MNS	-∞	-3.22	-1.70	-0.43	-0.01	0.07
Huntington's disease against GC	-∞	-2.27	-1.20	-0.32	0.00	0.07
G8 against MNS	-∞	-8.38	-3.97	-0.55	0.45	0.37
G8 against GC	-∞	-2.73	-1.17	-0.08	0.14	0.08

A, American pedigree; V, Venezuelan pedigree; T, total.

A 99% confidence interval (in this case one-sided) for the distance between the Huntington's disease locus and G8 is computed as the value of θ at which the likelihood is 100 times less than its maximum value, that is 2 log units less than 8.53. The lod score at 10 centimorgans (cM) is 6.52. Thus the 99% confidence interval is 0–10 centimorgans. One can also obtain a confidence interval based on the total number of non-cross-overs. However, in these Huntington's disease pedigrees this approach is not feasible because at-risk individuals cannot be determined as cross-overs.

The lod scores for both Huntington's disease and G8 against MNS (located between 4q28 and 4q31) and GC (located between 4q11 and 4q13) suggest that these latter markers are not close to either the Huntington's disease (or G8) locus. These

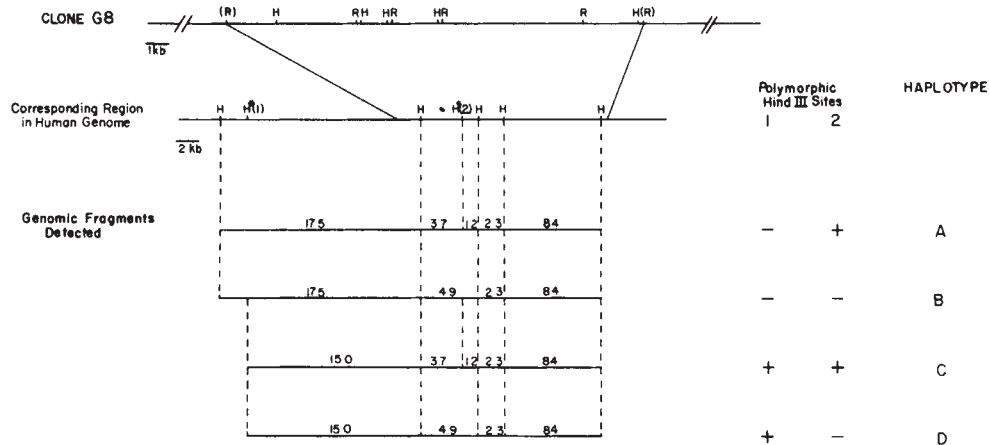
results eliminate a substantial portion of chromosome 4 as the possible region containing the Huntington's disease gene.

Implications

The discovery of a DNA marker genetically linked to the Huntington's disease locus has profound implications both for investigations of the basic gene defect and for clinical care. Certain questions must be resolved, however, in order to clarify the clinical applicability of this finding, considering the benefits and hazards involved in presymptomatic testing²⁰.

The data in this study were obtained from only two families, although they were from different ethnic backgrounds. Further study is required to determine whether Huntington's disease is

Fig. 4 Restriction map of the G8 insert and the corresponding region of chromosome 4. The restriction maps of the G8 insert and corresponding region of the genomic DNA were determined as described in the text. (H, *Hind*III sites; R, *Eco*RI sites). The polymorphic *Hind*III sites 1 and 2 are labelled H*(1) and H*(2). The two *Eco*RI sites bordering the insert are placed in parentheses as they were created during the cloning procedure¹⁵ and are not present in genomic DNA. Restriction sites in the Charon 4A vector arms are not shown. The genomic fragments generated by *Hind*III digestion that are detected by hybridization to the G8 probe are shown for each haplotype. The major allele due to polymorphism at *Hind*III site 1 is 17.5 kb fragment resulting from the absence of cleavage (-). The minor allele, as 15.0 kb fragment, results from the presence of cleavage (+) at this site. The major allele due to polymorphism at *Hind*III site 2 occurs when the site is present (+) yielding fragments of 3.7 and 1.2 kb. The minor allele in this case is a 4.9 fragment occurring when site 2 is absent (-). The haplotypes were named as follows: A, major allele at site 1, major allele at site 2; B, major allele at site 1, minor allele at site 2; C, minor allele at site 1, major allele at site 2; and D, minor allele at site 1, minor allele at site 2. It should be noted that the gel pattern for the combination AD is identical to that for BC. These can only be distinguished if the linkage phase of the alleles at sites 1 and 2 is determined by typing immediate relatives of the individual in question.



genetically heterogeneous (that is, produced by a variety of different mutations at different loci). Heterogeneity is unlikely given the exceedingly low mutation rate for the illness and clear migration patterns of the Huntington's disease gene from Europe to other parts of the World, but this possibility must certainly be tested¹.

It is equally important to assess the value of this marker for diagnosis and future research by determining how closely linked it is to the Huntington's disease locus. No obligate crossover between G8 and Huntington's disease has yet been found, and an estimate of the 99% confidence interval for the genetic distance separating the two loci extends to 10 cM. Refining the accuracy of this estimate requires testing many more individuals. Even if the G8 marker ultimately proves to be of limited utility for diagnosis, it will now be possible to use chromosome-specific cloning techniques to generate many more markers in the same region and to determine their linkage relationships to the Huntington's disease locus^{7,21}. The development of many markers closely linked on chromosome 4 will increase the potential for presymptomatic testing.

If genetic heterogeneity can be excluded, and G8 is sufficiently close to the Huntington's disease locus, it can be used in a linkage analysis to provide presymptomatic and prenatal determination of Huntington's disease gene carriers in that fraction of families that are appropriately informative. Sufficient family members must be available for analysis and must be segregating the G8 marker for the test to be successful. We are presently attempting to maximize the degree of heterozygosity, and therefore the informativeness of this locus, by searching for additional RFLPs with the G8 probe.

If the G8 marker maps quite close to the Huntington's disease gene then linkage disequilibrium may exist between the two loci. If this were the case, then it is possible the G8 marker could lead to a screening test for Huntington's disease carriers within known Huntington's disease pedigrees rather than a linkage test. It should be noted, however, that in the two families

tested here, one American and one Venezuelan, the Huntington's disease gene segregates with a different G8 haplotype in each case (A and C respectively).

Clearly caution must be exercised in considering clinical applications of the G8 marker, but its application to Huntington's disease research is immediate. The discovery of a marker linked to the Huntington's disease gene makes it feasible to attempt the cloning and characterization of the abnormal gene on the basis of its map location. Understanding the nature of the genetic defect may ultimately lead to the development of improved treatments. Furthermore, this study demonstrates the power of using linkage to DNA polymorphisms to approach genetic diseases for which other avenues of investigation have proved unsuccessful. It is likely that Huntington's disease is only the first of many hereditary autosomal diseases for which a DNA marker will provide the initial indication of chromosomal location of the gene defect.

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- Hayden, M. R. *Huntington's Chorea* (Springer, New York, 1981).
- Wexler, N. S. *Genetic Counseling: Psychological Dimensions* (ed. Kessler, S.) 190-220 (Academic, New York, 1979).
- Bruyn, G. W. *Prog. Brain Res.* **55**, 445-464 (1982).
- Martin, J. B. *Nature* **299**, 205-206 (1982).
- Pericak-Vance, M. A. *et al. Adv. Neurol.* **23**, 59-72 (1979).
- Volkers, W. S. *et al. Ann. hum. Genet.* **44**, 75-79 (1980).
- Housman, D. & Gusella, J. F. *Molecular Genetic Neuroscience* (eds Schmitt, F. O., Bird, S. J. & Bloom, F. E.) 415-424 (Raven, New York, 1982).
- Botstein, D., White, R. L., Skolnick, M. & Davis, R. *Am. J. hum. Gen.* **32**, 314-331 (1980).
- Kan, Y. W. & Dozy, A. M. *Lancet* **ii**, 910 (1978).
- Solomon, E. & Bodmer, W. F. *Lancet* **i**, 923 (1979).
- Murray, J. M. *et al. Nature* **300**, 69-71 (1982).
- Wexler, N. S. *et al. Cytogenet. Cell Genet.* (in the press).

- Shoulson, I. & Fahn, S. *Neurology* **29**, 1-3 (1979).
- Gusella, J. *et al. Cytogenet. Cell Genet.* (in the press).
- Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. *Cell* **15**, 1157-1174 (1978).
- Gusella, J. F., Tanzi, R., Anderson, M. A., Ottina, K. & Watkins, P. in *Banbury Report 14: Recombinant DNA Applications to Human Disease* (eds Caskey, C. T. & White, R. L.) 261-266 (Cold Spring Harbor Laboratory, New York, 1983).
- Ott, J. *Am. J. hum. Genet.* **26**, 588-597 (1974).
- Shows, T. B., Sakaguchi, A. Y. & Naylor, S. L. *Adv. hum. Genet.* **12**, 341-452 (1982).
- Naylor, S. L., Gusella, J. & Sakaguchi, A. Y. *Cytogenet. Cell Genet.* (in the press).
- Wexler, N. S. in *Progress in Medical Genetics*, Vol. 7 (ed. Barton Childs) (Saunders, Philadelphia, in the press).
- Gusella, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 2829-2833 (1980).
- Naylor, S. L. *et al. J. molec. Biol.* **98**, 503-517 (1983).
- Gusella, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 5239-5243 (1979).
- Varsanyi-Breiner, A. *et al. Gene* **7**, 317-334 (1979).

Localization of the *c-abl* oncogene adjacent to a translocation break point in chronic myelocytic leukaemia

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The human c-abl oncogene maps within the region (q34-qter) of chromosome 9 which is translocated to chromosome 22, the Philadelphia (Ph') chromosome, in chronic myelocytic leukaemia (CML). The position of the Ph' chromosomal break point is shown to be variable and, in one CML patient, has been localized immediately 5' of, or within, the c-abl oncogene. A DNA restriction fragment corresponding to this site has been molecularly cloned and shown to represent a chimaeric fragment of DNA from chromosomes 9 and 22.

HUMAN cellular sequences designated oncogenes have been identified using retroviral transformation-specific sequences as molecular probes. Evidence for the potential involvement of such oncogenes in human cancer is accumulating rapidly. For instance, human tumour-derived DNA sequences, isolated on the basis of their ability to transform mouse cells phenotypically in tissue culture, have been shown to correspond to particular classes of cellular oncogenes¹⁻⁵. More recently, amplification of specific cellular oncogenes in tumour cells has been demonstrated⁶⁻⁸ and other human oncogenes have been shown to be involved in highly specific chromosomal translocations characteristic of particular classes of human neoplasia⁹⁻¹³.

The Abelson strain of murine leukaemia virus (MuLV) represents a recombinant between Moloney MuLV and *c-abl*, a cellular oncogene of mouse origin¹⁴. In contrast to other oncogenic retroviruses, Abelson MuLV transforms lymphoid cells *in vitro*¹⁵ and induces a rapid B-cell lymphoma *in vivo*¹⁶. We have previously reported the molecular cloning of the *v-abl* homologue (*c-abl*) from a human cosmid library¹⁷. Human *v-abl* homologous sequences were shown to be dispersed over a total region of around 32 kilobases (kb) and to contain a minimum of six introns. Extensive homology in nucleic acid and amino acid sequence was demonstrated between the tyrosine phosphorylation region of *c-abl* and corresponding regions of the *v-src*, *v-yes* and *v-fes/fps* family of viral oncogenes, as well as a more distant relatedness to the catalytic chain of the mammalian cyclic AMP-dependent protein kinase¹⁸. These findings suggest that each of these oncogenes was probably derived from a common progenitor and may represent members of a diverse, but highly related, family of cellular genes encoding protein kinases.

The human *c-abl* oncogene was initially mapped to the long (q) arm of chromosome 9 (ref. 19). This observation was of interest in view of the frequent involvement of chromosome 9 in the translocation with chromosome 22, t(9; 22), characteristic of chronic myelocytic leukaemia (CML)²⁰. Analysis of a series of rodent-human somatic cell hybrids containing either the 9q+ or the 22q- (Philadelphia, Ph') chromosome demonstrated the translocation of human *c-abl* from chromosome 9 to the Ph' chromosome⁹. We show here that in one Ph'-positive CML patient, the chromosomal break point maps within 14 kb 5' of the human *c-abl* oncogene. Such a break point could not be identified at this position in the DNAs of two other patients, arguing that the chromosomal break in Ph'-positive CML can occur at variable sites on chromosome 9. These findings strongly implicate the human *c-abl* oncogene in Ph'-positive CML.

Search for the t(9; 22) junction site

To investigate the possibility that the chromosome 9 break point in the t(9; 22) may be localized either within or in close proximity to the human *c-abl* oncogene, high molecular weight DNA was isolated from biopsy samples of the spleens of three CML patients, each of which contained high percentages of leukaemic cells. In addition, DNA was isolated from an erythroleukaemic cell line, K562, established from a CML patient in blast crisis²¹. Each DNA was digested with *Bam*HI and subjected to Southern blot analysis. As shown in Fig. 1a, the restriction enzyme patterns of K562 DNA and DNA of patient 0319129 were indistinguishable from those of control DNA, when hybridized to a previously described¹⁷ *v-abl* probe, P_{1.7}. Similarly, no differences in restriction enzyme patterns could be detected in the other two CML DNAs, or in any of the DNAs using other restriction enzymes or probes isolated from different regions of the human *c-abl* locus (not shown).

Chromosomal walking

As no rearrangements could be detected in the *v-abl* homologous cellular DNA region encompassed by our cosmid clones, a probe was prepared corresponding to an 0.4-kb *Hind*III-*Eco*RI fragment (0.4 HE) mapping within the 5' domain of the human *c-abl* locus (Fig. 2) and was used to screen a previously described human cosmid library²². Although recombinants representative of approximately 10 times the complete human genome were analysed, no positive clones were obtained. Analysis of total human DNA revealed that a *Bam*HI fragment of ~14.5 kb hybridized to the 0.4 HE probe. To characterize this restriction fragment further, a library of size-fractionated *Bam*HI-digested total human DNA was constructed in Charon 30. Screening of the library with the 0.4 HE probe revealed seven positive recombinant phage clones. All positive plaques were extremely small and turbid. The insert of one of these positives was subcloned into pBR328 (p5'-*c-abl*) and subjected to detailed restriction enzyme analysis as shown in Fig. 2b. No sequences in the 14.5-kb fragment hybridized to any of a series of previously described¹⁷ probes corresponding to the complete *v-abl* genome.

As indicated in Fig. 2b, an 0.2-kb probe was prepared from the 5' terminus of p5'-*c-abl* by digestion with *Bam*HI and *Eco*RI. This probe hybridized to a *Hind*III fragment, of ~9.5 kb, in all DNAs examined, suggesting that sequences immediately 5' to the newly cloned region are non-rearranged (Fig. 1c, lanes 3-7). As expected, a fragment of 14.5 kb was

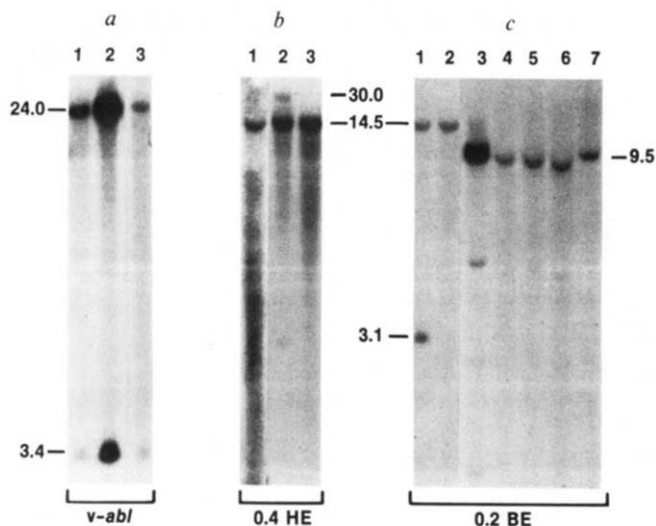


Fig. 1 Identification of DNA sequences mapping within and 5' of the human *c-abl* locus. *a*, *Bam*HI digest of 10 μ g of DNA from human cell line A673 (lane 1), K562 (lane 2) and patient 0319129 (lane 3). *b*, *Bam*HI digest of 10 μ g DNA from CML 02120185 (lane 1), CML 0319129 (lane 2) and CML 0311068 (lane 3). *c*, *Bam*HI digest of DNA from patient 0319129 (lane 1), 0311068 (lane 2), *Hind*III digest of DNA from cell line K562 (lane 3), CML 02120185 (lane 4), CML 0319129 (lane 5), CML 0311068 (lane 6) and cell line A204 (lane 7). Molecular probes including a previously described *v-abl* probe (*v-abl* P_{1.7})¹⁷, 0.4 HE and 0.2 BE (see Fig. 2) are indicated at the bottom of the figure. Frozen spleen tissue was obtained through the NIH Resources and Logistics Branch, DCCP. Patient 0319129 was a 26-yr-old male, patient 0218105 was a 57-yr-old male and patient 0311068 was a 67-yr-old female. Cell line K562, pedigree 722, (GM 5372) was obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey. DNA was prepared from tissue and cell lines according to Jeffreys and Flavell³⁰. DNA was digested with restriction enzymes, electrophoresed on 0.75% agarose gels and blotted according to the method of Southern³¹. Isolation of probes, hybridization and washings, to 0.3 $\times$ SSC at 65 $^{\circ}$ C, were as described elsewhere^{17,32}. Molecular weights of the hybridizing fragments (kb) are as indicated.

detected in *Bam*HI-digested normal human DNA, while in DNA of CML patient 0319129, an additional *Bam*HI restriction fragment of $\sim$ 3.1 kb (Fig. 1c, lane 1) was identified. Similarly, the 0.4-kb HE probe isolated from the 5' region of *c-abl* hybridized with a 14.5-kb *Bam*HI fragment in the other two CML DNAs (Fig. 1b). In DNA from CML patient 0319129, however, an extra *Bam*HI fragment of $\sim$ 30 kb was visible (lane 2). The most likely explanation for these findings is that one allelic copy of DNA sequences 5' to *c-abl* in CML patient 0319129 DNA is normal while the second is rearranged; these appear to be present in about a 1:1 molar ratio.

To define more accurately the region where DNA of patient 0319129 differs from the normal region, probes including an 0.52-kb *Eco*RI fragment (0.52 E) and an 0.76-kb *Eco*RI-*Hind*III fragment (0.76 HE) (see Fig. 2b) were prepared from p5'-*c-abl*, corresponding to the approximate region of rearranged sequences in the DNA of patient 0319129. As shown in Fig. 3, extra DNA fragments were found with these probes on restriction enzyme digestion of DNA 0319129 with *Bgl*II, *Xba*I, *Sst*I and *Hind*III. The 9.5-kb 5' *Hind*III fragment, which hybridized to the 0.52-kb *Eco*RI probe, seemed to be present in a normal quantitative level, as did the 2.0-kb fragment hybridizing to the 0.76-kb HE probe. A rearrangement thus appeared to have taken place within the 1.25-kb *Hind*III fragment since, in addition to the normal 1.25-kb *Hind*III fragment, a fragment of 2.2-kb can be identified.

Molecular cloning

As human *c-abl* is translocated to the Philadelphia chromosome (22q⁻) in CML, we reasoned that the transposed sequences in DNA of CML patient 0319129 should include most of the 14.5-kb *Bam*HI fragment; the 5' sequences of this fragment would presumably remain on chromosome 9, linked to sequences from chromosome 22, thus accounting for the 3.1-kb *Bam*HI fragment (Fig. 1c, lane 1). The 9.5-kb *Bgl*II fragment (Fig. 3b) would thus represent the chromosome 22/9 chimaeric fragment localized at the Ph' translocation break point; the 6.0-kb *Bgl*II fragment (Fig. 3b) would contain sequences from chromosomes 9 and 22, on the 9q+ chromosome. Alternatively, the differences between the DNA of patient 0319129 and control DNA could be the result of DNA rearrangements, deletions or insertions having no connection with the t(9; 22) Ph' translocation.

To distinguish between these possibilities, DNA from CML patient 0319129 was digested with *Bgl*II, size fractionated in

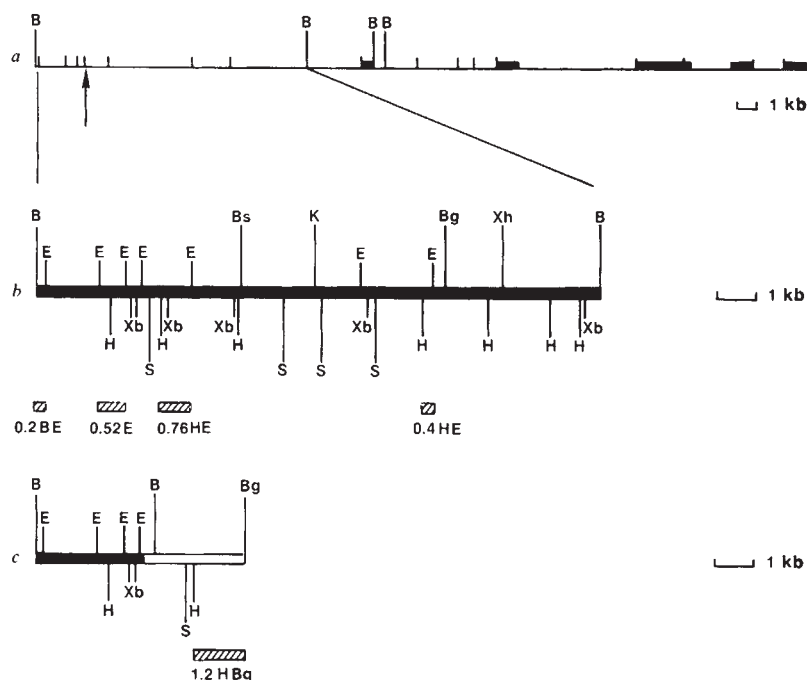


Fig. 2 Restriction enzyme analysis of p5'-*c-abl* and a part of pBglII 9q+. The position of p5'-*c-abl* in relation to the *v-abl* hybridizing region in the human *c-abl* locus is indicated in *a*. *Eco*RI sites are marked by small vertical lines; *v-abl* homologous sequences are indicated by solid bars. The vertical arrow indicates the break point in the DNA of CML 0319129. A restriction map of p5'-*c-abl* is shown in *b*; probes used in this study, indicated as hatched boxes, are shown beneath the map. The pBglII 9q+ fragment in *c* is a subclone of a 6.0-kb *Bgl*II fragment isolated from CML patient 0319129; in *b* and *c* the *Bgl*II site within 200 bp of the 5' *Bam*HI site and a *Hpa*I site in between these two restriction sites are not shown. The solid bar indicates sequences from chromosome 9 while the open bar represents sequences of the p5'-*c-abl* and *Bgl*II-9q+ sequences was according to previously described methods³³. Restriction enzyme maps are based on hybridization data and on results of double digestions of individual fragments isolated from low melting point agarose gels. Restriction enzymes include: *Bam*HI (B), *Bgl*II (Bg), *Bst*EII (Bs), *Hind*III (H), *Sst*I (S), *Xba*I (Xb), *Xho*I (Xh), *Eco*RI (E) and *Kpn*I (K).

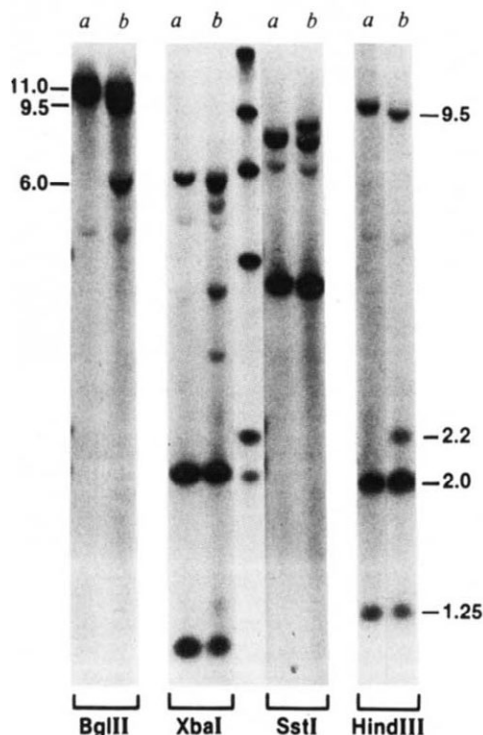


Fig. 3 Restriction enzyme analysis of the DNA of CML patient 0319129. Restriction enzymes used and the molecular weights of the fragments in the *Bgl*II and *Hind*III digestions are as indicated. 32 P-labelled *Hind*III digestion fragments of λ DNA in the centre of the figure serve as a molecular weight marker for the *Xba*I and *Sst*I digestions. Southern blots were hybridized with a mixture of the 0.52 E and 0.76 HE probes (see Fig. 2b), as described in Fig. 1 legend. DNA of the cell line A673 is shown in lanes a; DNA of CML 0319129 is in lanes b.

the 6–7-kb range, and a library constructed in *Bam*HI-digested Charon 30 phage arms. Three positive clones were identified on screening with the 0.2-kb BE probe. Because each of the positive clones contained an additional inserted *Bgl*II fragment, some *Bgl*II sites had remained intact, allowing the subcloning of most of the 6.0-kb *Bgl*II fragment by *Bgl*II–*Hpa*I digestion into plasmid ORF²³. Colonies containing this recombinant plasmid were visible only on a two- to threefold longer incubation at 37 °C than neighbouring non-positive colonies. This particular DNA region may thus contain sequences that inhibit bacterial growth, possibly explaining our failure to detect these sequences in cosmid libraries. As shown in the restriction enzyme map of this fragment (Fig. 2c), the *Eco*RI site 3' of the 0.52-kb *Eco*RI fragment is still present. In contrast, an *Sst*I site immediately 3' of it and all other restriction enzyme sites to the 3' are either missing or different from those found in the same region in p5'-c-*abl*. These findings localize a putative break point in p5'-c-*abl* between the *Eco*RI and *Sst*I sites. Sequences 5' of this point in the 6.0-kb *Bgl*II fragment hybridize to p5'-c-*abl*; sequences 3' of it do not (results not shown).

To determine the origin of the 6.0-kb *Bgl*II fragment, a 1.2-kb *Hind*III–*Bgl*II probe (1.2 HBg) was prepared (Fig. 2c). As shown in Fig. 4, using stringent washing conditions, this probe hybridized to a 5.0-kb *Bgl*II fragment in DNA of the human cell line A204 (lane 2), but not to sequences in mouse DNA (lane 1) or in rodent–human somatic cell hybrids containing human chromosome 9 (lane 4)⁹. In an independent hybrid (PgMe-25Nu), however, containing chromosome 22 as its only human component⁹, a 5.0-kb *Bgl*II fragment was detected (lane 3). Moreover, using the same probe both 5.0-kb and 6.0-kb *Bgl*II fragments were identified in the DNA of patient 0319129 (lane 6). The 1.2 HBg probe did not hybridize to sequences in the human–mouse somatic cell hybrid WESP-2A (Fig. 4, lane

Fig. 4 Chromosomal localization of the 3' domain of pBglII-9q+. High molecular weight DNAs were digested with *Bgl*II, electrophoresed on an 0.75% agarose gel, blotted and hybridized to the 1.2 HBg probe (see Fig. 2c) and included mouse (lane 1), A204 (lane 2), PgMe-25Nu (chromosome 22, lane 3), 10CB-23B (chromosome 9, lane 4), WESP-2A (chromosome 22q-, lane 5) and CML patient 0319129 (lane 6).

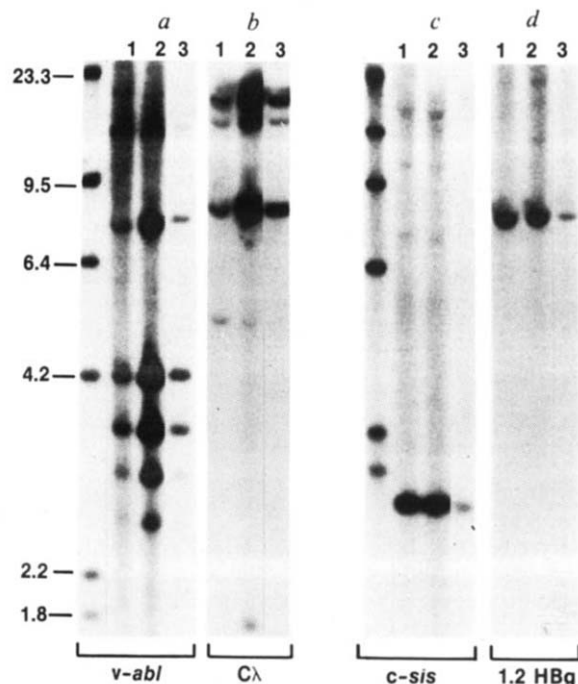
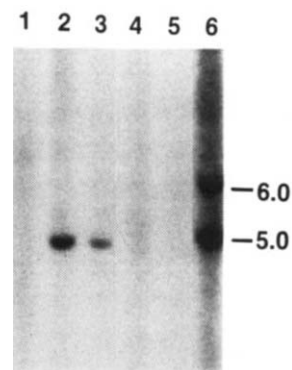


Fig. 5 Amplification of c-*abl* in the erythroleukaemic cell line K562. Control human DNA (10 μ g, lanes 1 in each panel) was run next to 10 μ g (lanes 2) and 2.5 μ g (lanes 3) K562 DNA. 32 P-labelled *Hind*III-digested λ DNA is included as a molecular weight standard for a and b (0.6% agarose gels) and for c and d (0.75% agarose gels). a, DNAs were digested with *Eco*RI and hybridized to a previously described v-*abl* probe, v-*abl* P_{1,7} (ref. 17). b, *Eco*RI-digested DNAs were hybridized to a *Bgl*II–*Hind*III probe, isolated from a human C_λ clone Hu λ 5 (ref. 25). c, *Bam*HI-digested DNA hybridized to a previously described²⁶ 1.7-kb *Bam*HI probe prepared from a human c-*sis* cosmid clone. d, *Bgl*II-digested DNAs hybridized to the 1.2 HBg probe (Fig. 2c).

5), which contains the Philadelphia chromosome in the absence of 9,q+ or 22. Thus, the 6.0-kb *Bgl*II fragment cloned from patient 0319129 DNA must contain, in addition to sequences from chromosome 9, sequences originating from the translocated region of chromosome 22.

Amplification of c-*abl*

Although the K562 cell line lacks a cytogenetically identifiable Ph' chromosome (ref. 24 and A. Hagemeijer, personal communication), it was reported to contain a small ring chromosome r(22), which is probably derived from a Ph' chromosome²⁴. Our results suggest that, at a minimum, K562 contains a chromosome(s) resembling the Ph' chromosome marker. As shown in Fig. 5d, using the 1.2 HBg probe, a non-amplified 5.0-kb *Bgl*II fragment was detected in K562 DNA. In contrast, human c-*abl* sequences are amplified at least fourfold in K562 DNA, as are

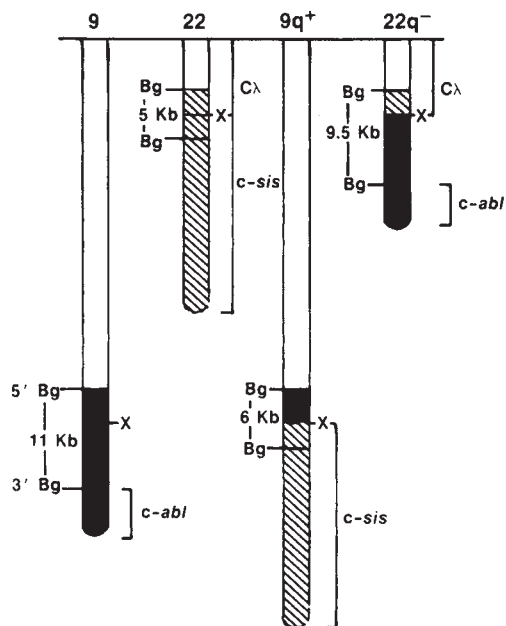


Fig. 6 Schematic representation of the t(9;22) in CML patient 0319129. The sizes of the *Bgl*II fragments detected with probes from chromosome 9 and 22 are indicated. Regions in black originate from chromosome 9; hatched regions are from chromosome 22. An X indicates the t(9;22) chromosomal break point. The human *c-sis* oncogene and the C_λ region on chromosome 22 are shown for purposes of orientation; human *c-sis* is localized on chromosome 22 (ref. 34) and is translocated to chromosome 9 in CML²⁶. C_λ is localized in band q 11 of chromosome 22 (ref. 10), and is not involved in the Ph' translocation (unpublished results).

sequences up to 17 kb upstream of the most 5' *v-abl* homologous *Eco*RI region (Fig. 1c). A 11.0-kb *Eco*RI fragment (Fig. 5a) that hybridizes to the *v-abl* P_{1.7} probe but has no linkage with the main human *c-abl* locus¹⁷ is non-amplified and thus serves as an internal control. The immunoglobulin light-chain constant region (C_λ)²⁵ is also amplified at least fourfold (Fig. 5b). This latter observation is consistent with the localization of human *c-abl* and C_λ sequences on the same amplification unit, presumably a part of the Ph' chromosome. In concordance with this conclusion, C_λ remains on the Ph' chromosome in the t9:22 (unpublished observations). In contrast, another human oncogene, *c-sis*, which is located on chromosome 22 and transferred to chromosome 9 in the Ph' translocation²⁶, is non-amplified (Fig. 5c). Finally, the fact that the 5.0-kb *Bgl*II fragment normally localized on chromosome 22, is non-amplified in K562, supports the assumption that it is translocated to chromosome 9 in this cell line.

Conclusions

The present findings establish that in one of three CML patient DNAs examined, the Ph' chromosomal break point is localized

within 14 kb of the most 5' *v-abl* homologous region. Because the position of the most 5' exon in human *c-abl* is not known, the possibility that the t(9;22) break point may even map within a coding region of the human *c-abl* locus cannot be excluded. We have not localized the break points in the DNAs of the two other CML patients or in DNA of the cell line WESP-2A, containing the 22q- chromosome. Our findings, however, establish that the site of the Ph' translocation break point is variable and suggest that break points in the latter DNAs may be located 5' of the ~80 kb of cloned DNA encompassing the human *c-abl* locus. Similarly, the t(8;14) translocation in Burkitt lymphoma, involving the human *c-myc* oncogene, does not appear to involve a single site^{10-12,27}. The orientation of human *c-abl* on chromosome 9, with its 5' region towards the centromere of the chromosome (Fig. 6), is established by the fact that the break point has been found 5' to the human *c-abl* locus.

K562 cells lack a cytogenetically identifiable Ph' chromosome despite their derivation from a CML patient in blast cell crisis²¹. However, genes on either side of the break point in the Ph' chromosome, *c-abl* and C_λ , are not only present in this cell line, but are amplified. In contrast, no amplification was found in the case of *c-sis*, normally on chromosome 22 but translocated to chromosome 9 in Ph'-positive CML, or a DNA region translocated to chromosome 9 corresponding to a restriction fragment containing the t(9;22) break point isolated from a CML patient. The amplification seems to be specific; neither human *c-fes*, on chromosome 15 (ref. 19), nor *c-fms*, on chromosome 5 (ref. 28), is amplified (data not shown). The finding that *c-abl* is amplified in K562 can be explained in two ways: it can reflect the fact that these cells were grown in tissue culture, possibly resulting in specific chromosomal aberrations and/or amplifications; alternatively, the *c-abl* may have been amplified during the blast crisis of the patient, the overproduction of the *c-abl* gene product being the cause of the disease. The relative amplification of a region of DNA, which includes both *c-abl* and C_λ sequences, suggests that the two are nearly contiguous in this cell line.

We have previously demonstrated the translocation of a small piece of chromosome 9 to chromosome 22 in CML patients carrying the Ph' chromosome⁹. The size of this fragment was calculated to be less than 5,000 kb, suggesting a potential role of the *c-abl* oncogene in CML. The isolation, in the present study, of a region of DNA from a CML patient that contains a chromosomal break point which is localized within 14 kb of the *c-abl* locus, the specific amplification of *c-abl* sequence in the K562 cell line, and the finding that *c-abl* is translocated to chromosome 22 in complex Ph' translocations²⁹ further implicate the *c-abl* oncogene in chronic myelocytic leukaemia.

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- Pulciani, S. *et al.* *Nature* **300**, 539-542 (1982).
- Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637-3640 (1982).
- Tabin, C. J. *et al.* *Nature* **300**, 143-149 (1982).
- Taparowsky, E. *et al.* *Nature* **300**, 762-765 (1982).
- Shimizu, K. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 2112-2116 (1983).
- Collins, S. & Groudine, M. *Nature* **298**, 679-681 (1982).
- Dalla-Favera, R., Wong-Staal, F. & Gallo, R. *Nature* **299**, 61-63 (1982).
- Alitalo, K., Schwab, M., Lin, C., Varmus, H. E. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1707-1711 (1983).
- de Klein, A. *et al.* *Nature* **300**, 765-767 (1982).
- Taub, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 7837-7841 (1982).
- Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cary, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1982-1986 (1983).
- Dalla-Favera, R. *et al.* *Science* **219**, 963-967 (1983).
- Sheer, D. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 5007-5011 (1983).
- Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. *Cell* **22**, 777-785 (1980).
- Rosenberg, N., Baltimore, D. & Scher, C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1932-1936 (1975).
- Abelson, H. T. & Rabstein, L. S. *Cancer Res.* **30**, 2213-2222 (1970).

- Heisterkamp, N., Groffen, J. & Stephenson, J. R. *J. molec. appl. Genet.* **2**, 57-69 (1983).
- Groffen, J., Heisterkamp, N., Reynolds, F. Jr. & Stephenson, J. R. *Nature* **304**, 167-169 (1983).
- Heisterkamp, N. *et al.* *Nature* **299**, 747-750 (1982).
- Rowley, J. D. *Nature* **243**, 290-293 (1973).
- Lozzio, C. B. & Lozzio, B. B. *Blood* **45**, 321-334 (1975).
- Groffen, J., Heisterkamp, N., Grosfeld, G., van de Ven, W. & Stephenson, J. R. *Science* **216**, 1136-1138 (1982).
- Weinstock, G. M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 4432-4436 (1983).
- Klein, E. *et al.* *Int. J. Cancer* **18**, 421-431 (1976).
- Hieter, P. A., Hollis, G. F., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. *Nature* **294**, 536-540 (1981).
- Groffen, J. *et al.* *J. exp. Med.* **158**, 9-15 (1983).
- Stanton, L. W., Watt, R. & Marcu, K. B. *Nature* **303**, 401-406 (1983).
- Groffen, J. *et al.* *Nucleic Acids Res.* (in the press).
- Bartram, C. R. *et al.* *Nature* **306**, 277-280 (1983).
- Jeffreys, A. J. & Flavell, R. A. *Cell* **12**, 429-439 (1977).
- Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
- Bernards, R. & Flavell, R. A. *Nucleic Acids Res.* **8**, 1421-1533 (1980).
- Groffen, J., Heisterkamp, N., Blennerhassett, G. & Stephenson, J. R. *Virology* **126**, 213-227 (1983).
- Swan, D. C. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 4691-4696 (1982).

Immunoglobulin heavy-chain class switching in a pre-B cell line is accompanied by DNA rearrangement

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Switching of an Abelson virus-transformed mouse lymphoid cell line from immunoglobulin μ to $\gamma 2b$ heavy-chain synthesis in vitro is accompanied by loss of DNA sequences between the J_H and $C_{\gamma 2b}$ gene segments, and thus cannot be explained by differential RNA processing. The light-chain loci of both μ - and $\gamma 2b$ -producing cell clones are in the embryonic configuration, which indicates that class switching can occur in pre-B cells.

AN individual B lymphocyte produces antibodies of a single specificity. Antibodies, or immunoglobulins, are composed of two identical heavy (H) and two identical light (L) chains. The specificity of the antibody is determined by the combination of the variable (V) regions of the H and L chains. The constant (C) region of the H chain defines the immunoglobulin class. During ontogeny, a haematopoietic stem cell with commitment to the B-lymphocyte series undergoes a stage of differentiation termed the pre-B cell¹. Pre-B cells produce H chains of the μ type but no L chain². The gene for the μ chain, as for all immunoglobulin chains, is generated in ontogeny. The variable part, formed by rearrangement of the embryonic DNA segments V_H , D_H and J_H (reviewed in ref. 3), and the nearby segment for the constant region of the μ chain (C_μ) together constitute the gene for the μ chain, which is transcribed into nuclear RNA. The introns are excised from this nuclear RNA to form a mRNA that consists of contiguous sequences encoding the μ chain. A pre-B cell becomes a B lymphocyte when it begins to synthesize L chain and inserts the complete immunoglobulin into the cell membrane. Most small B lymphocytes synthesize two immunoglobulin classes, IgM and IgD, simultaneously. This does not violate the 'one cell-one specificity' rule, as long as the V region is the same on the μ and δ chains and the same L chain is associated with the H chains. The gene segment for the δ chain constant region (C_δ) is adjacent 3' to C_μ . It was suggested, and some evidence for this has been provided, that in the case of cells synthesizing both μ and δ , there is a long nuclear RNA transcript that includes the V, D, J, C_μ and C_δ gene segments^{4,5}. The δ -chain mRNA would then be made by excising the C_μ together with the other introns.

Some small B lymphocytes express IgM together with other immunoglobulin classes (in the mouse, for example: IgG1, IgG2a, IgG2b, IgG3, IgE and IgA)^{6,7}. It has been proposed that an RNA splicing mechanism similar to that for μ and δ is responsible for such double isotype expression⁸. As the DNA segments encoding the various constant regions are spread out over 200 kilobases (kb)⁹, the nuclear RNA would have to be quite long, for example, ~180 kb for the ϵ chain. In any event, for B lymphocytes which have become plasma cells and secrete large amounts of immunoglobulin of classes other than IgM, a deletion of DNA sequences is found (reviewed in ref. 3). The missing DNA segment contains C_μ and all other sequences located between C_μ and the constant-region gene that is expressed by a particular plasma cell. Therefore, at least for plasma cells, RNA splicing is not involved.

We have studied the H-chain class switch in the Abelson virus-transformed lymphoid cell line, 18-81, which was derived from mouse bone marrow¹⁰. We have previously found that this line contains cells that produce μ or $\gamma 2b$ H chain or no immunoglobulin¹¹. The studies reported here were designed to determine whether the μ -positive cells switch to $\gamma 2b$ production or whether the μ - and $\gamma 2b$ -positive cells are derived exclusively

from cells which do not produce any immunoglobulin. The finding of a H-chain class switch from μ to $\gamma 2b$ *in vitro* allowed us to study this event at the DNA level. Contrary to a previous report¹², we find that C_μ is deleted whenever μ -positive cells switch to $\gamma 2b$ production.

Another question of interest is when, during ontogeny, does the H-chain class switch occur? The $\gamma 2b$ -producing cells, like the μ -producing cells, do not synthesize any L chain¹¹, which suggests that pre-B cells can switch. We have investigated the DNA arrangement of the L-chain loci and have confirmed this.

Heavy-chain expression in single cells

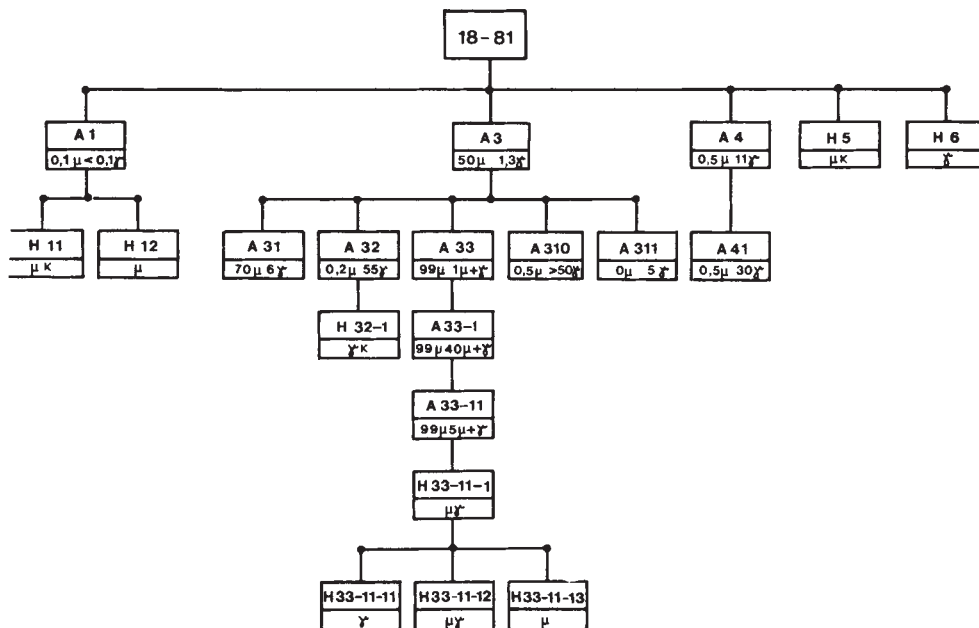
Most cells in the 18-81 cell line produce no H chain, others produce μ , and still others produce $\gamma 2b$. We also found a few cells which produce both μ and $\gamma 2b$ chains¹¹. Fusion of cells of this line to a myeloma producing no immunoglobulin yielded hybridomas producing μ or $\gamma 2b$ chain. Some hybridomas also expressed κ light chain^{11,13}.

To determine the potential for immunoglobulin expression and H-chain class switching by cells of this line, we examined many different subclones derived from clones representative of the various extremes of immunoglobulin production, that is, clones containing mainly immunoglobulin-negative cells, or a high percentage of μ -positive or $\gamma 2b$ -positive cells. Subclones were characterized by immunofluorescence at a time when they had expanded to ~10⁵ cells. Because the cellular composition of the subclones, like that of the original 18-81, is constantly changing, we often fused our subclones with a myeloma to obtain relatively stable hybridomas. The genealogy and composition of most of the clones presented here are shown in Fig. 1; the genealogy of clones that are not included can be deduced from the clone designation.

Cells can switch from μ to $\gamma 2b$ production

We demonstrated that cells synthesizing μ can switch to $\gamma 2b$ production, by subcloning clone A3. In this clone, 50% of the cells were μ -positive, 1% $\gamma 2b$ -positive, and 49% were immunoglobulin-negative. Subcloning by micromanipulation of single cells gave 98% cloning efficiency. Of the 128 daughter clones, 74% contained cells producing $\gamma 2b$. Among the $\gamma 2b$ -containing clones, 82 had predominantly μ cells, and 12 had predominantly $\gamma 2b$ cells with <0.5% μ cells. We refer to these 12 clones as $\gamma 2b$ clones although they contain some μ cells because we know that in this rare case, the μ chain produced is derived from the usually silent homologue¹⁴ and that the μ -producing cells represent a distinct cell population within the clone. Of 20 hybridomas derived from the subclone A32, 18 produced $\gamma 2b$ alone and 2 produced no H chain. From this number of hybridomas we recovered none producing μ . However, it is possible to clone out those μ -producing cells in a larger subcloning experiment¹⁴.

Fig. 1 Genealogy of selected subclones and hybridomas derived from the Abelson virus-transformed line 18-81. Abelson virus-transformed cells are designated 'A', their hybridomas 'H'. Subcloning was performed by limiting dilution¹¹ or by micromanipulation of single cells. Cytoplasmic μ , $\gamma 2b$ and κ chains were detected by immunofluorescence¹¹. The percentages of μ and $\gamma 2b$ cells in the 'A' lines are given below the subclone designation. Cell fusions were performed as described elsewhere¹¹ except for the A1 fusion. A1 is a bromodeoxyuridine-resistant, thymidine kinase-negative subclone of 18-81. In this case, both fusion partners could be selected against in media containing hypoxanthine-aminopterin-thymidine. The myeloma used for fusion, Ag8653 (ref. 20), contributes no immunoglobulin H or L chains to the hybridomas.



The percentage of $\gamma 2b$ cells decreases

Immunofluorescence analysis of the early 18-81 line showed about an equal percentage (10%) of μ - and $\gamma 2b$ -synthesizing cells. After fusion we obtained 55 hybridomas, of which 20 produced μ and 15 $\gamma 2b$. However, on subcloning of 18-81 after 1 yr in culture, only 3 of 240 subclones (~1%) were positive for $\gamma 2b$. Subcloning of the clone A3 which represents an early stage in 18-81 clonal development (see below) gave 12 $\gamma 2b$ subclones out of 133 (11%). Clearly, therefore, $\gamma 2b$ cells grow more slowly or die off earlier than μ cells.

DNA arrangements of μ and $\gamma 2b$ cells

Significant variability is observed in the percentage of 18-81 cells expressing one or the other of the H chain classes, both among subclones and within a given subclone with time in culture. This observation could be explained in one or more of the following ways: (1) The cell line can have a fixed DNA arrangement which is the same for all cells. The μ and $\gamma 2b$ chains expressed would be encoded on the same homologue and differential H-chain class expression would be brought about by, for example, differential splicing of a common nuclear RNA transcript. (2) The DNA is in a fixed arrangement, but both homologues are used, each for expression of a different H chain, the $\gamma 2b$ gene being formed, for example, by a switch rearrangement. (3) There is irreversible DNA rearrangement during clonal development, for example, the original 18-81 isolate would contain two C_μ alleles, one of which would be deleted on switching to $\gamma 2b$ production.

To differentiate among these possibilities, we have isolated cellular DNAs from various subclones, digested them with different restriction enzymes, and analysed by Southern blotting the DNA fragments containing immunoglobulin gene segments.

We found that on class switching in the A3 subclone, at least the one C_μ gene segment that was used for μ -chain expression was always deleted. The enzyme *Bam*HI cuts within the C_μ segment, so that two bands (9.4 and 11.2 kb) were found in embryonic DNA when hybridized with a μ cDNA probe (Fig. 2). As the enzyme also cuts between J_2 and J_3 , any rearrangement to form an active transcriptional VDJ unit with J_3 or J_4 or any deletion 3' to this restriction site will change the size of the 9.4-kb embryonic fragment.

The clone A3 which contains primarily μ -producing cells (with <2% $\gamma 2b$ -positive cells), and its μ -producing subclones A34, A35 and A36 have two copies of C_μ , as revealed by the 5.6-kb and 3.5-kb fragments (Fig. 2a). (Normally one would expect *Bam*HI fragments to be the same size or larger than the

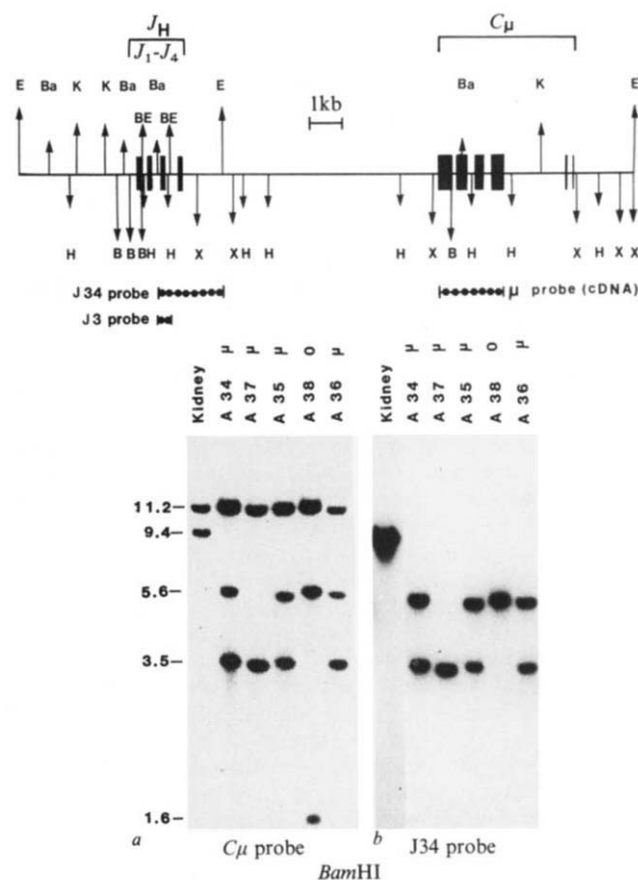


Fig. 2 Identification of the active allele. Top, restriction enzyme cleavage map of 18 kb of embryonic BALB/c mouse DNA that includes the J_H and C_μ gene segments. Ba, *Bam*HI; BE, *Bst*EII; B, *Bgl*II; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; X, *Xba*I. The locations of the restriction cleavage sites were provided by Blattner and are also given elsewhere^{9,21,22}. Bottom, Southern blot analysis²³ of DNA from 18-81-derived subclones. High molecular weight DNA was isolated²⁴ from the various cell lines, digested to completion with the different restriction enzymes (from BRL and Biolabs), electrophoresed in 0.8% agarose and transferred to nitrocellulose filters. The filters were hybridized with probes (indicated by $\bullet$) that had been labelled with 32 P-dCTP by nick-translation. The *Bam*HI-digested DNA was hybridized with a μ cDNA probe (a) or with the J34 probe (b). Plasmids containing the indicated probes were given by F. Blattner.

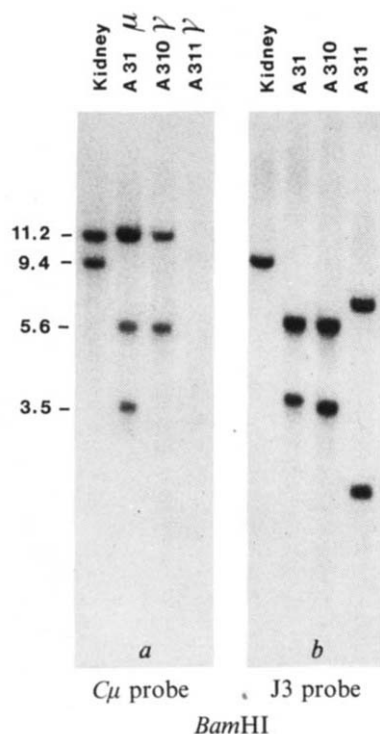


Fig. 3 The switch from μ to $\gamma 2b$ production in the 18-81 cell line is accompanied by deletion of one or both C_μ alleles. Southern blots of *Bam*HI-digested DNA were hybridized with either the C_μ or J3 probe as described in Fig. 2 legend.

9.4-kb embryonic fragments. However, deletions in the J - C_μ intron result in smaller fragments.) The 5.6-kb band is absent from subclone A37, which nevertheless contains 82% μ cells. Clone A38, which produces almost no immunoglobulin chain, has lost the J segment of the 3.5-kb band (Fig. 2b). (Only rarely were μ cells found, but in this case the μ chain produced is derived from the usually silent homologue¹⁴.) Clearly, therefore, the μ chain in clone A3 is made predominantly from the homologue represented by the 3.5-kb *Bam*HI fragment which, on switching to $\gamma 2b$ production, is then lost. The subclone A310 which contains mainly γ cells (0.5% μ cells) has lost the 3.5-kb fragment (Fig. 3a), as have seven other independent $\gamma 2b$ subclones. The hybridomas derived from $\gamma 2b$ clones also show loss of this C_μ copy. The $\gamma 2b$ -producing subclone A311 has lost both C_μ gene segments. Although subclones A310 and A311 had lost one or both C_μ alleles, respectively, they nonetheless retained two J_H alleles, as evidenced by hybridization of the *Bam*HI fragments (Fig. 3b) with a probe covering the J_3 segment ('J3 probe').

As the heavy-chain class switch in this line is accompanied by DNA deletion, one would predict that the 5' flanking sequences of the $\gamma 2b$ gene segment would be altered when bringing $C_{\gamma 2b}$ into closer proximity to *VDJ*. In clones A39 and A4, we detected DNA rearrangement involving the switch region of the $\gamma 2b$ segment. We base these conclusions on results from digestion with the enzymes *Sac*I, *Bgl*II, *Bgl*III and *Kpn*I (for restriction sites see Fig. 4), and hybridization with a $\gamma 2b$ cDNA probe. Figure 4 shows the *Bgl*III analysis for clone A32: DNA from the μ producer A31 shows the same bands as kidney DNA, while in A32, a $\gamma 2b$ producer, there is a new 12-kb band. This band also contains a J segment, as demonstrated by elution of the $\gamma 2b$ probe and rehybridization of the same filter with the J34 probe. We conclude, therefore, that the $\gamma 2b$ gene segment of clone A32 is localized on the same-sized DNA fragment as the *VDJ* segment.

μ and $\gamma 2b$ chains can use same *V* region

We have demonstrated that the μ and $\gamma 2b$ chains can use the same *VDJ* gene segment. The enzyme *Bst*EII cuts 5' and 3' to

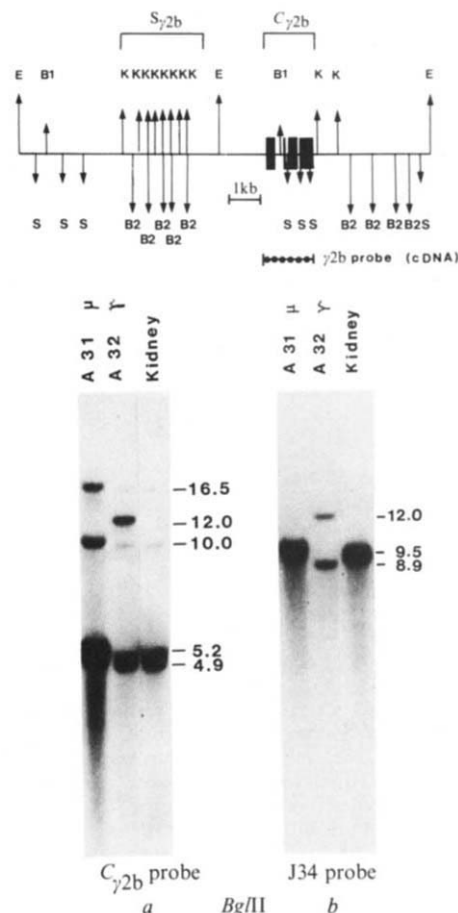


Fig. 4 The DNAs of $\gamma 2b$ cells show switch rearrangement. Top, restriction enzyme cleavage map of 13 kb of embryonic BALB/c mouse DNA that includes the $C_{\gamma 2b}$ switch rearrangement region ($S_{\gamma 2b}$). B1, *Bgl*II; B2, *Bgl*III; E, *Eco*RI; K, *Kpn*I; S, *Sac*I. Restriction enzyme sites are from refs 25-27. The *Bgl*II digests were hybridized with a $\gamma 2b$ cDNA probe (*Kpn*I-*Bam*HI fragment of the cDNA clone described in ref. 28)(a). After elution of the $\gamma 2b$ probe, the filter was rehybridized with the J34 probe (b).

J_2 and J_3 at the positions given in Fig. 2. Different V_H - D_H joining to J_2 or J_3 should create DNA fragments of different sizes which would be revealed with the J3 probe. The same two *VDJ* alleles, at the level of restriction enzyme mapping, are found in all 18-81 subclones, for example, in A32 (Fig. 5a). To define the active allele, we used hybridomas to correlate loss of a given restriction fragment with loss of immunoglobulin production. *Bst*EII DNA fragments of hybridomas H33-11-11, a $\gamma 2b$ producer, and H11, a μ producer, both show two identical bands (Fig. 5a). The 1.3-kb band is contributed by the myeloma Ag8653; the 5.1-kb band should contain the *VDJ* rearrangement, the only one present. The analogous situation is found for *Hind*III analysis (Fig. 5b). Analysis using the enzymes *Eco*RI, *Xba*I and *Bam*HI and hybridization with the J34 probe also reveals that hybridomas H11 and H33-11-11 contain only one *VDJ* gene segment. We conclude, therefore, that the μ and $\gamma 2b$ chains can use the same *V* region, a conclusion also reached by Alt *et al.*¹².

Light-chain loci in $\gamma 2b$ producers

We have shown previously that the $\gamma 2b$ cells of the 18-81 line, like the μ cells, do not synthesize any L chain¹¹. Pre-B cells do not synthesize any L chain because the L chain loci are in the embryonic configuration¹⁵. We found the same situation in the 18-81 cell line: the $\gamma 2b$ -producing cells, and hybridomas derived from them, had their loci for κ and λ chains in the embryonic configuration (Fig. 6). Cellular DNA was digested with the

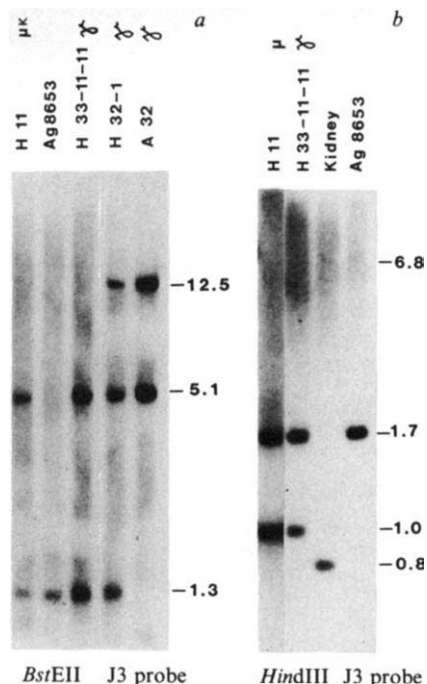


Fig. 5 The μ - and $\gamma 2b$ -producing cells in the 18-81 cell line use the same V region. Southern blot analysis of *BstEII*(a) and *HindIII*(b)-digested DNA was done as described in Fig. 2 legend except that *HindIII* digests were electrophoresed in 1.5% agarose. Filters were hybridized with the J3 probe. Refer to Fig. 2 for *BstEII* and *HindIII* sites in embryonic DNA.

restriction enzymes *EcoRI*, *XbaI* or *BamHI*. As shown in Fig. 6, V_{κ} rearrangement should create new 5' *XbaI*, *BamHI* and *EcoRI* restriction sites. Results for *XbaI*-digested DNA analysed with a J_{κ} probe are shown in Fig. 6a. Five subclones of line 18-81 were examined for κ -chain gene rearrangement; all show the same embryonic pattern (Fig. 6a, lanes 2-5, 7)—two bands of identical size to those seen in *XbaI*-digested kidney DNA (arrows). Fusion of the μ - (and also $\gamma 2b$ -) producing cells of 18-81 sometimes results in rearrangement and expression of a κ -chain gene (Fig. 6a, lane 6)^{11,13}. Two $\gamma 2b$ subclones (Fig. 6a, lanes 5 and 7) and a $\gamma 2b$ hybridoma derived from a fusion with the 18-81 line (lane 8) fail to express L chain and have undergone no rearrangement of their κ -chain genes. *EcoRI* and *BamHI* digests confirmed this (not shown). The λ -chain gene locus was also not rearranged in any of the cell lines tested (Fig. 6b). Thus, we conclude that the H-chain class switch can occur at the pre-B cell level.

One can ask whether the H-chain class switch observed in the 18-81 line is physiological. Pre-B cells synthesizing γ chains have not been identified in murine or human lymphopoietic tissues; this could be due to the low frequency of μ pre-B cells switching to γ . However, human acute lymphocytic leukaemia cells having pre-B cell characteristics that synthesize various

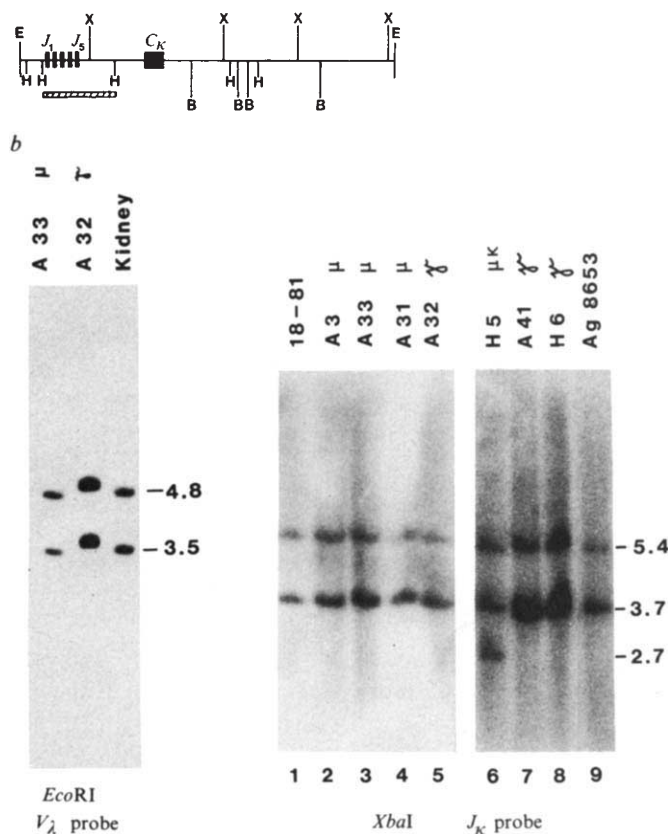


Fig. 6 The switch from μ to $\gamma 2b$ can occur before κ or λ light-chain gene rearrangement. a, Restriction enzyme cleavage map of a 15-kb *EcoRI* fragment containing the J_{κ} and C_{κ} gene segments that was isolated from NZB mouse embryo DNA²⁹. The phage clone Ch4A-N κ 3 was given by N. Hozumi. The restriction enzyme sites are identical to those published for BALB/c embryo DNA^{30,31}. For Southern blot analysis, *XbaI*-digested DNA was hybridized with the 2.9-kb *HindIII* fragment (■ at top) which includes $J_{\kappa}1-J_{\kappa}5$. b, Southern blot analysis of *EcoRI*-digested DNA hybridized with a probe containing an embryonic $V_{\lambda 1}$ gene segment (0.9-kb *XbaI*-*HindIII* fragment). This probe was isolated and given by S. Weiss. $V_{\lambda 1}$ (3.5 kb) and $V_{\lambda 2}$ (4.8 kb) cross-hybridize and both can be seen in the embryonic configuration (see, for example, ref. 32).

H-chain classes have been described¹⁶. Class switching at the pre-B cell level does not, of course, exclude switching later in B-cell differentiation¹⁷⁻¹⁹.

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- Raff, M. D., Megson, M., Owen, J. J. T. & Cooper, M. D. *Nature* **259**, 224-226 (1976).
- Burrows, P. D., Lejeune, M. & Kearney, J. F. *Nature* **280**, 838-841 (1979).
- Marcu, K. B. *Cell* **29**, 719-721 (1982).
- Wabl, M. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 6793-6796 (1980).
- Maki, R. *et al. Cell* **24**, 353-365 (1981).
- Abney, E. R., Cooper, M. D., Kearney, J. F., Lawton, A. R. & Parkhouse, R. M. E. *J. Immun.* **120**, 2041-2049 (1978).
- Pernis, B., Fornì, L. & Luzzati, A. L. *Cold Spring Harb. Symp. quant. Biol.* **41**, 175-183 (1976).
- Yaoita, Y., Kumagai, Y., Okumura, K. & Honjo, T. *Nature* **297**, 697-699 (1982).
- Shimizu, A., Takahashi, N., Yaoita, Y. & Honjo, T. *Cell* **28**, 499-506 (1982).
- Siden, E. J., Baltimore, D., Clark, D. & Rosenberg, N. E. *Cell* **16**, 389-396 (1979).
- Burrows, P. D., Beck, G. B. & Wabl, M. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 564-568 (1981).
- Alt, F. W., Rosenberg, N., Casanova, R. J., Thomas, E. & Baltimore, D. *Nature* **296**, 325-331 (1982).
- Riley, S. C., Brock, E. J. & Kuehl, W. M. *Nature* **289**, 804-806 (1981).
- Wabl, M. R., Beck-Engeser, G. B. & Burrows, P. D. *Proc. natn. Acad. Sci. U.S.A.* (submitted).
- Maki, R., Kearney, J., Paige, C. & Tonegawa, S. *Science* **209**, 1366-1369 (1980).

- Vogler, L. B. *et al. Nature* **290**, 339-341 (1981).
- Nossal, G. J. V., Warner, N. & Lewis, H. *Cell. Immun.* **2**, 41-53 (1971).
- Kearney, J. F., Cooper, M. D. & Lawton, A. R. *J. Immun.* **116**, 1664 (1976).
- Wabl, M. R., Fornì, L. & Loo, F. *Science* **199**, 1078-1080 (1978).
- Kearney, J. F., Radbruch, A., Liesegang, B. & Rajewsky, K. *J. Immun.* **123**, 1548-1550 (1979).
- Liu, C. P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. *Science* **209**, 1348-1353 (1980).
- Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676-683 (1980).
- Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
- Gross-Bellard, M., Dudet, P. & Chambon, P. *Eur. J. Biochem.* **36**, 32-38 (1973).
- Tucker, P. W., Marcu, K. B., Newell, N., Richards, J. & Blattner, F. R. *Science* **206**, 1303-1306 (1979).
- Yaoita, Y. & Honjo, T. *Nature* **286**, 850-853 (1980).
- Kataoka, T., Miyata, T. & Honjo, T. *Cell* **23**, 357-368 (1981).
- Tucker, P. W., Marcu, K. B., Slightom, J. L. & Blattner, F. R. *Science* **206**, 1299-1303 (1979).
- Hozumi, N., Hawley, R. G. & Murialdo, H. *Gene* **13**, 163-172 (1981).
- Max, E. E., Seidman, J. G. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3450-3454 (1979).
- Sakano, H., Hüppi, K., Heinrich, G. & Tonegawa, S. *Nature* **280**, 288-294 (1979).
- Brack, C., Hiram, M., Lenhard-Schuller, R. & Tonegawa, S. *Cell* **15**, 1-14 (1978).

Time-delayed photoelectric effect

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The Einstein photoelectric relation¹ initially explained the photoelectric effect,

$$h\nu = T_{\max} + E_b$$

where $h\nu$ is the energy of a photon incident on a material, E_b is the binding energy of the electron to be excited, and $T_{\max}$ is the maximum kinetic energy of the emitted electron. This work has generated considerable research²⁻⁵ because of its multidisciplinary interest. We describe here photoelectric emission (PE) experiments using very low-intensity nanosecond light pulses with energies near the PE threshold. Signal correlated, time-delayed pulses of emitted electrons were observed for single light pulses incident on a photosensitive material. However, the energies of the delayed emitted electrons were not correlated to the wavelength of the incident light, which is not consistent with Einstein's relation. At higher incident light intensities, the delayed pulses were not observed. These pulses were not due to electrical noise because they disappeared when the laser light was blocked from entering the cell. They were not due to single photon luminescence from a particular species because the pulses were observed using another experimental apparatus with an alternate source of nanosecond light. We now describe these delayed pulses and give a preliminary interpretation of them.

The photosensitive material was a semitransparent (Cs)Na₂KSb (p-type) photocathode in a RCA 7265 photomultiplier tube (PMT). Pulses from a Quanta Ray dye laser, pumped by a Quanta Ray DCR Nd:YAG laser, were directed through a stainless steel 316 cell. The pulses were 5–6 ns wide, with a wavelength of 556 nm. The cell was evacuated to a pressure between 10^{-4} and 5×10^{-5} torr. The PMT viewed the central portion of the cell in a direction perpendicular to the laser beam. The signal processing system consisted of a Tektronix R7912 transient digitizer, with a 7A19 amplifier, a 7B92A time base and a PDP-11 computer. The time base was triggered using an electrical pulse, synchronized with the Q-switch. During preliminary experiments, a low-level broadband signal (red and IR) was observed, with a pulse shape similar to the laser pulse. This signal was due to weak luminescence from the wall of the cell. The scattered 556 nm laser light was filtered using a long pass dichroic filter, with a 50% cutoff at 620 nm and a blocking factor of 200. Interference filters were used to block the 556 nm light by another factor of 2×10^4 and to define the desired frequency bandwidth of the luminescence signal.

Typical single shot experiments performed at 710 and 631 nm are shown in Fig. 1 (we define a shot as a PMT signal arising from a single laser pulse). The incident light intensities were equal at these two wavelengths (within 40%). At the shorter wavelength, the PMT output was a single regularly-shaped pulse (Fig. 1a). At the longer wavelength, the initial pulse was irregularly shaped and was followed by many small pulses (Fig. 1b).

The origin of the delayed pulses can be understood by examining the generally accepted theory of semiconductors. Photons are absorbed and electrons are promoted into the conduction band. Carrier-carrier and carrier-lattice interactions change the initial distribution of carrier energies in less than a few picoseconds⁶. Electrons reaching the surface with sufficient energy to overcome the surface barrier are emitted. The carriers with insufficient energy to escape, relax via carrier-lattice inter-

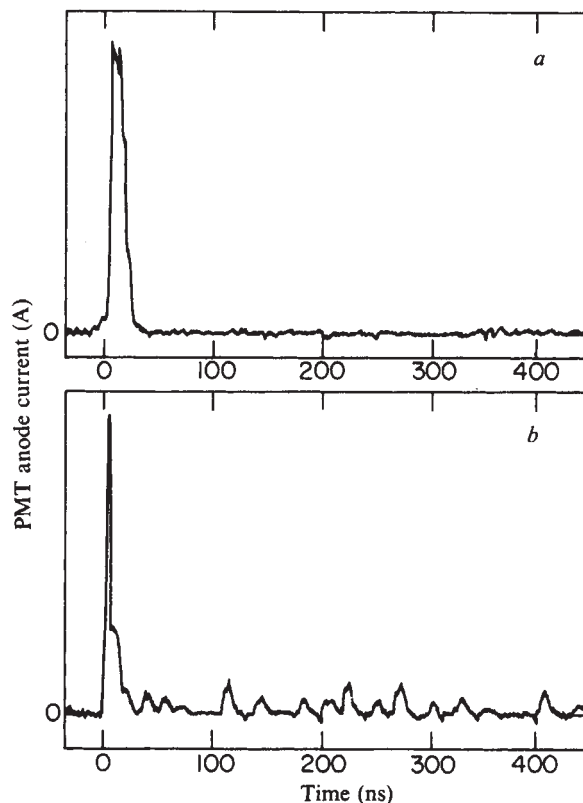


Fig. 1 PMT anode current (in arbitrary units) as a function of time and wavelength of incident light: a, 631 nm; b, 710 nm. The voltage applied to the PMT was 1,800 V.

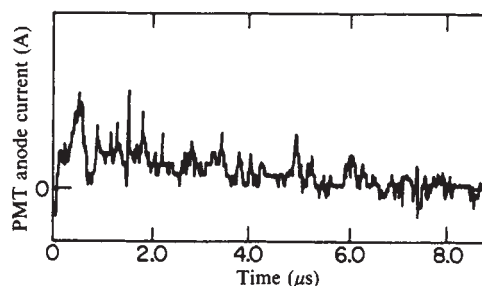


Fig. 2 A 10-μs trace of the PMT anode current as a function of time. The initial pulse is not shown.

actions. Within several picoseconds of removing the incident light, the carrier energy distribution reaches local equilibrium, which can be described by the lattice temperature⁶. These carriers remain in or near the conduction band until they either recombine with holes or are emitted. As the wavelength of incident light approaches the PE threshold, a smaller fraction of the excited minority carriers is emitted in the initial pulse than at shorter wavelengths. The traces in Fig. 1 are consistent with this explanation because at the longer wavelength, a smaller fraction of the electrons are emitted in the initial pulse, while a larger fraction of the carriers remain in the conduction band, available for emission at a later time.

The delayed pulses are either single emitted electrons or cascades of emitted electrons from the photocathode. The pulses occur at variable times and differ in magnitude by as much as a factor of five in one shot. The time of occurrence and the magnitude of the delayed pulses also change from shot to shot. The number of electrons corresponding to the pulse in Fig. 1a was estimated to be $300 (\pm 50\%)$ electrons. This number is

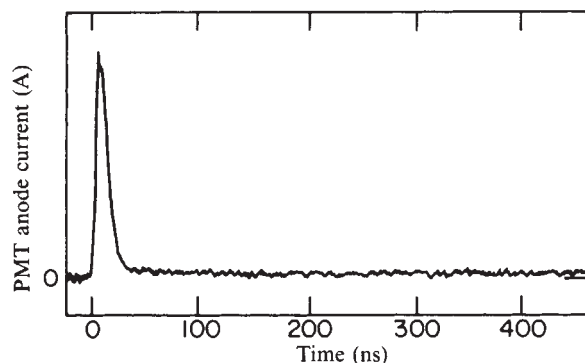


Fig. 3 A trace of PMT anode current versus time resulting from averaging 200 shots.

calculated from

$$N_e = (I_p \times \text{FWHM}) / (e^- \times G_{\text{PMT}})$$

where N_e is the number of electrons emitted, I_p is the peak anode current per pulse, FWHM is the full width at half maximum of the laser pulse, e^- is the charge of an electron and G_{PMT} is the gain of the PMT.

Since the maximum cathode current of the delayed pulses was 0.1 times the cathode current of the pulse shown in Fig. 1a, then the upper limit for the number of electrons in the delayed pulses was 30. It was not possible to calculate an accurate value for the number of electrons in a delayed pulse because of the FWHM of the delayed pulses was limited by the response time of the PMT (5 ns at 1,800 V).

When a signal trace of 10 μs duration was recorded in similar conditions to those for Fig. 1b, the height of the delayed pulses was observed to decrease over 6–7 μs (Fig. 2). As the lifetime range of minority carriers in semiconductors is of the order of 2 ms to 0.1 μs (ref. 7), the occurrence of the delayed pulses over 6–7 μs is consistent with the conclusion that the pulses are due to the excess minority carriers. In addition, the occurrence of the pulses is consistent with a mechanism in which the pulses are initiated by events which transfer sufficient energy to an electron(s) to enable it to escape from the surface. A higher energy carrier could be emitted or it could interact with another carrier(s) to produce a cascade of emitted electrons. Two possible pulse initiating events are carrier–lattice interactions and carrier–carrier interactions.

The delayed emission of electrons occurs while carriers in the conduction band are in a local equilibrium described by the temperature of the lattice, which, to a good approximation, constitutes an infinite sink. However, the total number of electrons generated in the conduction band by a single pulse is small, and a small system may not be truly chaotic, but a function of initial conditions. Nonetheless, whenever experiments were performed a large number of times and the signals averaged, the traces obtained did not display abnormal behaviour of the observable (Fig. 3). Therefore an average of such systems constitutes a canonical ensemble, to a good degree of approximation.

This time-delayed photoelectric emission, which occurs at very low intensities and long wavelengths of incident light, was not due to noise or single photon luminescence. Because the semiconductor studied was a thin film, the effects observed are of particular interest to those studying the photoelectric properties of semiconductor surfaces. The observation of signal-correlated emission, occurring after the minority carriers in the semiconductor have relaxed to a distribution characterized by the lattice temperature, indicates that the emission is photoelectric rather than thermionic. It also indicates that the energies of the emitted electrons are not correlated to the wavelength of the incident light. This type of PE does not obey Einstein's photoelectric relation.

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1. Einstein, A. *Ann. Phys.* **17**, 132 (1905).
2. Cardona, M. & Ley, L. *Topics appl. Phys.* **26** (1978).
3. Dobrzynski, L. (ed.) *Handbook of Surfaces and Interfaces* Vol. 1 (Garland STPM, New York, 1978).
4. Feuerbacher, B., Fitton, B. & Willis, R. F. (eds) *Photoemission and the Electronic Properties of Surfaces* (Wiley, Toronto, 1978).
5. Fiermans, L., Vennik, J. & Dekeyser, W. (eds) *Nato Adv. Stud. Inst.* **B32**, (1978).
6. Leheny, R. L., Shah, J., Fork, R. L., Shank, C. V. & Migus, A. *Solid St. Commun.* **31**, 809 (1979).
7. Davies, D. A. *Waves, Atoms and Solids* 288 (Langman Group, New York, 1978).

The 1/f distribution for tuneable oscillators

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An interesting explanation of the prevalence of noise whose spectral dependence on frequency f is like $f^{-\alpha}$ with $\alpha \approx 1$ has been given by Montroll and Shlesinger¹. They have remarked that the log normal distribution, in leading order, distributes a random variable x according to x^{-1} . Given this distribution they go on to explain, following van der Ziel² (see also ref. 3), how a 1/f power spectrum is a consequence. The simplicity of their argument is compelling, and leads us to observe here that in fact the 1/f distribution arises quite naturally in (at least) one other way.

Consider a large, fixed assembly of oscillators of unit mass, each of which can assume a different frequency. Let $P_1(\omega)$ be the distribution of frequencies among the oscillators. We require that this distribution be normalized:

$$\int_a^b P_1(\omega) d\omega = 1 \quad (1)$$

where the integration is over a physically dictated range of frequencies. If $P_1(\omega)$ varies as ω^{-1} , we need $a > 0$ and $b < \infty$.

We assume that there are many weakly coupled oscillators with fluctuating energies. We characterize the state of an oscillator by its action, which is an adiabatic invariant⁴. Suppose that an oscillator of frequency ω has the probability $P_2(J|\omega)$ of having an action J . Again we normalize:

$$\int_0^\infty P_2(J|\omega) dJ = 1 \quad (2)$$

Now let

$$P(\omega, J) = P_1(\omega)P_2(J|\omega) \quad (3)$$

and introduce the informational entropy⁵

$$S = - \int_0^\infty \int_a^b P \ln P d\omega dJ \quad (4)$$

We require that S be a maximum subject to equations (1) and (2), and that the total energy be constant. As the number of oscillators remains fixed, this last condition is that the mean energy of all the oscillators should be constant. The mean energy of an oscillator (if it is approximately linear) is

$$\langle E \rangle = \int_0^\infty \int_a^b \omega J P(\omega, J) d\omega dJ \quad (5)$$

The indicated calculation can readily be carried out with Lagrange multipliers, and we obtain

$$\begin{aligned} P_1(\omega) &= N/\omega, \\ P_2(J|\omega) &= \beta\omega e^{-\beta\omega J} \end{aligned} \quad (6)$$

where $N \ln(b/a) = 1$. The total probability P is the canonical distribution of energy ωJ , with $\beta = 1/\langle E \rangle$. With period $\tau \equiv 2\pi/\omega$, we find from $P_1(\omega)$ a distribution $P_1(\tau) \propto 1/\tau$. But if τ is the characteristic time for a random process, then by the argument given by van der Ziel² and Montroll⁶.

An important feature in the formalism is the constancy of the mean of the product of two or more statistical variables. Consider a system that produces a quantity Q at a rate R . Suppose the production rate is constant for time interval Δt during which an amount ΔQ is produced and that then R is changed discontinuously. We treat Δt and R as statistical variables and introduce the probabilities of finding the values R and Δt on a given cycle, $P_1(\Delta t)$ and $P_2(R|\Delta t)$. The formalism just described applies directly with $P = P_1 P_2$ and with the average over cycles of ΔQ fixed at the value $\langle \Delta Q \rangle$; the average is taken with respect to P . When we maximize entropy for fixed $\langle \Delta Q \rangle$, we obtain $P_1(\Delta t) = N/\Delta t$, in the notation used above. If Δt is a characteristic time for a random process, then, as above, $P_1(\Delta t)$ gives a $1/f$ power spectrum. If the parameter R is distributed so that, for some of the intervals, it assumes the value zero, but otherwise it maintains the same $P_2(R|\Delta t)$ in the remaining intervals, a distribution like $1/(\Delta t)^\alpha$, with α between zero and one, is obtained.

Finally, we note that for systems subject to the more general constraint that $\langle 6[\Delta t]^\mu R^\nu \rangle$ be constant, for an arbitrary function 6, we find that $P_1(\Delta t) = N(\Delta t)^{-\mu/\nu}$. In this case, we obtain a power spectrum proportional to $f^{-2+\mu/\nu}$.

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1. Montroll, E. W. & Shlesinger, M. F. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3380–3383 (1982).
2. van der Ziel, A. *Physica* **16**, 359–372 (1980).
3. Dutta, P. & Horn, P. M. *Rev. mod. Phys.* **53**, 497–516 (1981).
4. Landau, L. D. & Lifshitz, E. M. *Mechanics*, 154–158 (Pergamon, Oxford, 1960).
5. Khinchin, A. I. *Mathematical Foundations of Information Theory* 3 (Dover, New York, 1957).
6. Montroll, E. W. & Shlesinger, M. F. *J. stat. Phys.* **32** (2) (1983).

Molecular layering of water at surfaces and origin of repulsive hydration forces

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The short-range forces between hydrophilic surfaces in water determine the behaviour of many diverse systems such as the stability of colloidal dispersions^{1,2} and soap films³, the swelling of clays⁴ and the interactions of biological membranes^{5–8} and macromolecules⁹. So far, all experimental measurements of these forces have indicated that they are repulsive and decay monotonically with distance out to separations of up to ~ 6 nm. These forces, variously termed 'structural' or 'hydration' forces, arise from the energy needed to dehydrate interacting surfaces which contain ionic or polar species. Here we have measured, in some detail, the short-range hydration force between two molecularly smooth surfaces of mica containing hydrated potassium ions. We find that while the hydration force is overall repulsive it is not monotonic at separations ≤ 1.5 nm but exhibits oscillations, that is, alternating maxima and minima with a mean periodicity of 0.25 ± 0.03 nm, roughly the diameter of the water molecule. These results rationalize apparently irreconcilable observations on clay–water systems and go some way towards clarifying the origin and nature of hydration forces.

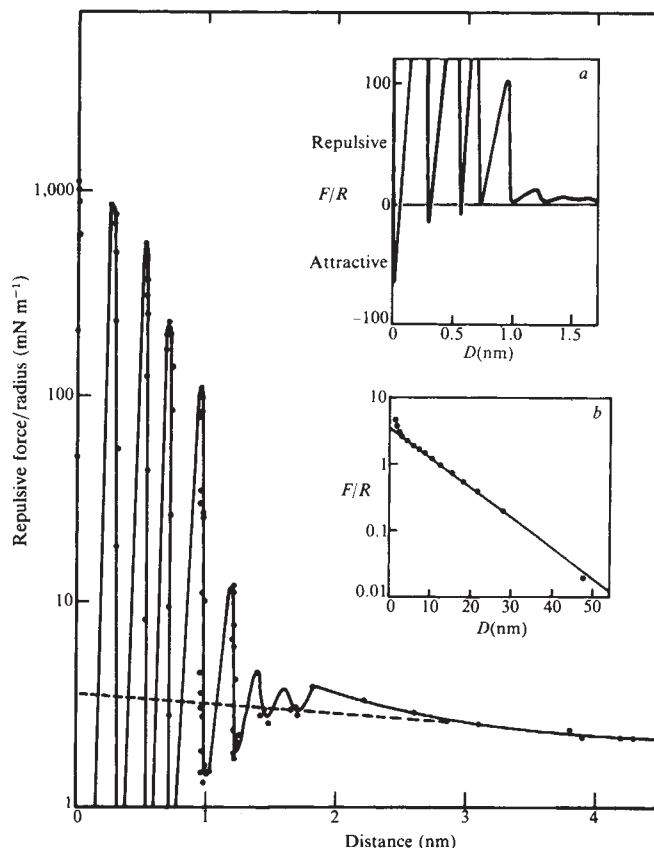


Fig. 1 Measured force F between two curved mica surfaces of initial radius $R \approx 1$ cm as a function of distance D in 10^{-3} M KCl solution (the plotted normalized force F/R is equivalent to $2\pi E$, where E is the interaction energy per unit area of two flat surfaces at the same separation D (ref. 34)). Inset *a*, force law at small separations, below 2 nm, plotted on a linear scale. Inset *b*, force law at large separations, beyond 2 nm, where the solid line (and dashed line in main figure) is the theoretical electrostatic double-layer force for a surface potential of -78 mV. The high distance resolution (± 0.03 nm) obtained in these experiments was achieved by coating the back surface of the mica with a thick silver layer which gave a high reflectivity of about 0.98, resulting in very sharp interference fringes. Under the influence of steep repulsive forces at small separations, the flattening of the surfaces also aided the increased accuracy over measurements at larger separations.

Hydration forces measured between mica^{10,11} and silica^{12,13} surfaces have shown that the repulsive force decays monotonically with an exponential distance dependence of characteristic length ~ 1 nm. For the case of mica at very close separations, below 1–1.5 nm, the repulsion increases much more steeply¹⁴. Experimental studies on soap films³ and lipid bilayers^{7,15} also show a steep short-range exponential repulsion—similar to that found for mica below ~ 1.5 nm.

We have previously reported on the nature of these forces between two curved mica^{10,11} and bilayer-coated mica¹⁵ surfaces in aqueous electrolyte solutions, where at separations below ~ 2 nm the repulsive pressures can exceed 100 atm. In these studies the forces were measured from the deflection of a single-cantilever spring. However, this method does not allow accurate measurement of these very large forces because of tilting and shearing of the opposing surfaces. To overcome this problem, we have modified the original force measuring apparatus¹⁰ by replacing this spring with a variable stiffness, double-cantilever spring, similar to that used in earlier measurements of Van der Waals forces¹⁶. This double spring allows more accurate measurement of both attractive and repulsive forces over a range of six orders of magnitude.

Figure 1 shows the short-distance force law measured between two mica surfaces in 10^{-3} M KCl solution at $pH = 5.5$. In these solution conditions it has previously been shown¹¹ that the mica

surfaces are partially covered with hydrated potassium ions at separations ≥ 3 nm, where the repulsive force is accurately described by diffuse electrical double-layer theory (Fig. 1 and inset *b*). However, at closer distances a strong repulsive hydration force prevents attractive Van der Waals forces from pulling the surfaces into adhesive contact (at $D=0$) as would be expected from the DLVO theory^{17,18}. Below 1.5 nm the force law exhibits oscillations of rapidly increasing amplitude but remains entirely within the repulsive regime until the last three, close-in, minima which lie below the $F=0$ axis (Fig. 1, inset *a*). Unfortunately, due to the very high repulsive pressures generated at distances below 1 nm, the curved mica surfaces flatten elastically and the forces at the maxima cannot be accurately measured. The depths of the minima can, however, be accurately determined irrespective of deformation¹⁹, as can the positions of the oscillations, and the periodicity of 0.25 nm was observed to be reproducible to ± 0.03 nm in three experiments.

These results have direct implications for theories of surface interactions. They indicate that the breakdown of continuum theories such as electrostatic (double-layer) forces and Van der Waals (Lifshitz) forces in very thin aqueous films is mainly due to solvent structural effects rather than, for example, discrete ion effects or dielectric saturation. Theoretical studies and computer simulations in which the molecular structure is specifically taken into account always predict oscillatory behaviour²⁰⁻²⁴, which has also been recently measured in both non-polar^{25,26} and polar²⁷ organic liquids, indicating the generality of the phenomenon.

On a more practical level these results suggest an explanation to a hitherto unresolved problem in soil science: X-ray diffraction studies of dispersed clay platelets similar to mica (such as, montmorillonite in NaCl solution) show that stable aggregates can form with water interlayers of typical thickness 0.25 and 0.55 nm, corresponding roughly with one and two layers of water in between the sheets²⁸. The mechanism involved in the formation of these aggregates has, until now, been a mystery as it has also been shown that on forcing Na-montmorillonite platelets together they are strongly repelled by a monotonically increasing hydration force similar to that observed between mica surfaces^{4,29,30}. However, from the results presented here we note (Fig. 1, inset *a*) that in spite of the large repulsive forces there are three deep minima (with negative interaction energies) at contact and at separations of 0.28 ± 0.03 and 0.56 ± 0.03 nm, in very good agreement with clay systems. It therefore seems most likely that dispersed platelets can only come into these minima by lateral sliding rather than by face-to-face approach. Experimental observation of aggregated platelets do, in fact, indicate lateral growth rather than stacking³¹, in agreement with our results.

Measurements of hydration forces between silica surfaces^{12,13} and between surfactant^{3,15} and lipid⁷ bilayers in water do not indicate any oscillatory behaviour and it is likely that a smooth, rigid surface is a prerequisite for their occurrence. In particular, the fluid-like surfaces of bilayers and biological membranes are highly mobile due to the thermal motions of the hydrated lipid head-groups and to fluctuations in bilayer thickness, estimated to be of the order of a few tenths of a nanometre^{32,33}. These fluctuations should smear out the discrete oscillatory character of the force-law. From Fig. 1, because the oscillations are not symmetrical about the $F=0$ axis the smeared out interaction would be monotonically repulsive (with a roughly exponential decay length between 0.2 and 0.3 nm, as has been observed in lipid bilayer interactions⁷).

In conclusion, we propose that the origin of the strong, short-range repulsive hydration force seen in many different systems is the same, and is due primarily to the ordered layering of water molecules bound to hydrated surface groups. For smooth rigid surfaces the force-law is an oscillatory function of distance, while for micro-rough or fluid surfaces the oscillations are smeared out resulting in a purely monotonic repulsion.

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1. Eagland, D. In *Water: A Comprehensive Treatise* Vol. 5 (ed. Franks, F.) Ch. 1 (Plenum, New York, 1975).
2. Deryaguin, B. V. & Churaev, N. V. *Croatia chem. Acta* **50**, 187-195 (1977).
3. Clunie, J. S., Goodman, J. F. & Symons, P. C. *Nature* **216**, 1203-1204 (1967).
4. Viani, B. E., Low, P. F. & Roth, C. B. *J. Colloid Interface Sci.* (in the press).
5. Le Neveu, D. M., Rand, R. P., Parsegian, V. A. & Gingell, D. *Biophys. J.* **18**, 209-230 (1977).
6. Cowley, A. C., Fuller, N. L., Rand, R. P. & Parsegian, V. A. *Biochemistry* **17**, 3163-3168 (1978).
7. Lis, L. J., McAlister, M., Fuller, N., Rand, R. P. & Parsegian, V. A. *Biophys. J.* **37**, 657-665 (1982).
8. Dunic, J. T., Israelachvili, J. N., Ninham, B. W., Pashley, R. M. & Thorne, S. W. *FEBS Lett.* **129**, 193-196 (1981).
9. Rau, D. R., Lee, B. K. & Parsegian, V. A. *Biophys. J.* **41** (No. 2, Pt 2), 418a (1983).
10. Israelachvili, J. N. & Adams, G. E. *Nature* **262**, 774-776 (1976); *JCS Faraday Trans. I* **74**, 975-1001 (1978).
11. Pashley, R. M. *J. Colloid Interface Sci.* **83**, 531-546 (1981).
12. Peschel, G., Belouschek, P., Müller, M. M., Müller, M. R. & König, R. *Colloid Polym. Sci.* **260**, 444-451 (1982).
13. Rabinovich, Y. I., Derjaguin, B. V. & Churaev, N. V. *Adv. Colloid Interface Sci.* **16**, 63-78 (1982).
14. Israelachvili, J. N. & Pashley, R. M. in *Biophysics of Water* (ed. Franks, F.) 183-194 (Wiley, New York, 1982).
15. Pashley, R. M. & Israelachvili, J. N. *Colloids Surfaces* **2**, 169-187 (1981).
16. Israelachvili, J. N. & Tabor, D. *Nature phys. Sci.* **236**, 106 (1972); *Proc. R. Soc. A* **A331**, 19-38 (1972).
17. Derjaguin, B. V. & Landau, L. *Acta phys. chim. URSS* **14**, 633-662 (1941).
18. Verwey, E. J. W. & Overbeek, J. Th. G. *Theory of the Stability of Lyophobic Colloids* (Elsevier, Amsterdam, 1948).
19. Israelachvili, J. N., Perez, E. & Tandon, R. K. *J. Colloid Interface Sci.* **78**, 260-261 (1980).
20. van Megen, W. & Snook, I. K. *JCS Faraday Trans. II* **75**, 1095-1102 (1979); *J. chem. Phys.* **72**, 2907-2913 (1980).
21. Grimsom, M. J., Rickayzen, G. & Richmond, P. *Molec. Phys.* **39**, 61-73, 1455-1462 (1980).
22. Chan, D. Y. C., Mitchell, D. J., Ninham, B. W. & Pailthorpe, B. A. *JCS Faraday Trans. II* **76**, 776-784 (1980).
23. Jönsson, B. *Chem. Phys. Lett.* **82**, 520-525 (1981).
24. Christou, N. I., Whitehouse, J. S., Nicholson, D. & Parsonage, N. G. *Faraday Symp. chem. Soc.* **16**, 139-149 (1981).
25. Horn, R. G. & Israelachvili, J. N. *J. chem. Phys.* **75**, 1400-1411 (1981).
26. Christenson, H. K. *J. Chem. Phys.* **78**, 6906-6913 (1983).
27. Christenson, H. K. & Horn, R. G. *Chem. Phys. Lett.* **98**, 45-48 (1983).
28. Del Pennino, U. et al. *J. Colloid Interface Sci.* **84**, 301-309 (1981).
29. Norrish, K. *Disc. Faraday Soc.* **18**, 120-134 (1954).
30. Callaghan, I. C. & Ottewill, R. H. *Disc. Faraday Soc.* **57**, 110-118 (1974).
31. Quirk, J. P. in *Modification of Soil Structure* (eds Emerson, W. W., Bond, R. D. & Dexter, A. R.) Ch. 1 (Wiley, New York, 1978).
32. Büldt, G., Gally, H. U., Seelig, J. & Zaccari, G. *J. molec. Biol.* **134**, 673-691 (1979).
33. Lis, L. J., McAlister, M., Fuller, N., Rand, R. P. & Parsegian, V. A. *Biophys. J.* **37**, 667-672 (1982).
34. Derjaguin, B. V. *Kolloid Z.* **69**, 155-164 (1934).

Evidence for Oligocene-Middle Miocene abyssal circulation changes in the western North Atlantic

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Previous studies of benthic foraminiferal isotopic composition have demonstrated that a latest Eocene-earliest Oligocene benthic foraminiferal $\delta^{18}\text{O}$ increase occurred in the Pacific, Southern and Atlantic Oceans¹⁻⁹. A Middle Miocene $\delta^{18}\text{O}$ increase has been noted in the Pacific, Southern and South Atlantic Oceans^{1-3,7,10,11} and tentatively identified in the North Atlantic^{12,13}. Due to the incomplete nature of the North Atlantic stratigraphical record^{14,15}, however, the Oligocene to Middle Miocene isotopic record of this ocean is poorly understood. In the modern ocean, the North Atlantic and its marginal seas has a critical role in abyssal circulation, influencing deep- and bottom-water hydrography as far away as the North Pacific¹⁶⁻¹⁸. We now report oxygen isotope measurements on Oligocene to Middle Miocene (12-36 Myr BP) benthic foraminifera in the western North Atlantic which show two periods of enriched $\delta^{18}\text{O}$ values: early Oligocene and early Middle Miocene. These enriched intervals are interpreted as resulting, in part, from the build-up of continental ice sheets. The Oligocene to Middle

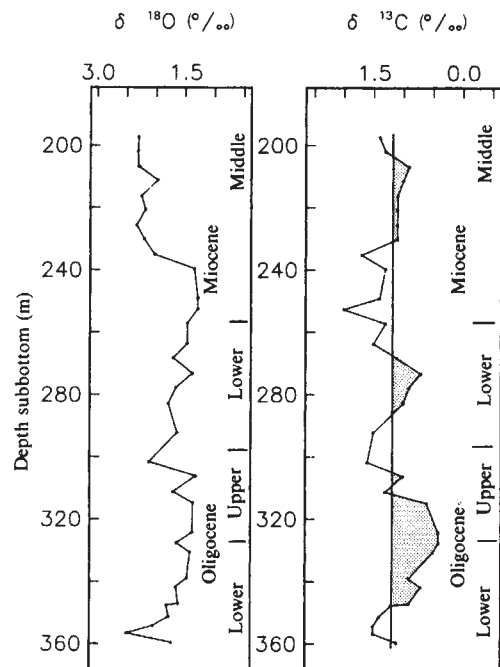


Fig. 1 Isotopic composition versus depth subbottom, Site 563, western North Atlantic. Solid line drawn through mean $\delta^{13}\text{C}$ of data in Table 1 (1.2‰) and intervals with depleted ^{13}C are indicated with stipples. Stratigraphical boundaries drawn using the zonations given in Table 1 and the time scales of Berggren *et al.*^{19,20}.

Miocene $\delta^{13}\text{C}$ record shows three cycles of enrichment and depletion of large enough magnitude to be useful for time-stratigraphical correlations. Within the biostratigraphical age resolution, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ records correlate with records from other oceans, helping to establish a useful Tertiary isotopic stratigraphy. An Atlantic-Pacific $\delta^{13}\text{C}$ contrast of 0.3–0.9‰ during the latest Oligocene to Middle Miocene (12–26 Myr BP) indicates North Atlantic deep- and bottom-water production analogous to modern North Atlantic deep water (NADW).

Recent rotary drilling at DSDP Site 563, western North Atlantic (38°39'N, 43°46'W; 3,796 m present depth; palaeodepths: early Oligocene, 2.2 km; late Oligocene, 2.9 km; Middle Miocene, 3.4 km), provided the first continuously deposited, biostratigraphically well-controlled, abyssal Atlantic record adequate for inter-ocean isotopic comparisons. Figure 1 shows the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ composition of earliest Oligocene (Zone P18) to late Middle Miocene (Zone N14) benthic foraminifera at North Atlantic Site 563. Biostratigraphical zonations are derived from planktonic foraminifera; absolute age control is obtained by interpolating between datum levels (Table 1) using the time scales of Berggren *et al.*^{19,20}. Detailed biostratigraphical zonal age assignments will be presented elsewhere (K.G.M., in preparation). Isotopic analyses were performed on mixed species of the benthic foraminiferal genera *Cibicides* and *Planulina*. These taxa were selected because they secrete calcite tests that are apparently constantly offset from $\delta^{18}\text{O}$ equilibrium by 0.65‰ (refs 21–25). Also, studies of Holocene core tops show that these taxa accurately reflect the distribution of $\delta^{13}\text{C}$ of ΣCO_2 in the modern oceans^{22–25}.

At Site 563, oxygen isotopic values are enriched in the Lower Oligocene ($\delta^{18}\text{O}$ = 1.6–2.5‰ between 341 and 360 m s.b. (metres subbottom); Table 1; Fig. 1) similar to lowermost Oligocene values reported elsewhere^{1–9}. Middle to Upper Oligocene values (301–341 m s.b.) are lower ($\delta^{18}\text{O}$ = 1.3–1.7‰). The significance of an enriched value in the uppermost Oligocene cannot be evaluated at present. Lower and lower-Middle Miocene $\delta^{18}\text{O}$ values range from 1.4 to 1.9‰. A major ^{18}O enrichment occurs between 235 and 239 m s.b. (Fig. 1) in the lower Middle Miocene (between Zones N9 and N10/11; 14.9–15.2 Myr BP).

Table 1 Oxygen and carbon isotopic data for Site 563, reported to PDB standard

Sample	Depth subbottom (m)	Zonal age* (Myr BP)	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
5-2 130–136 cm	197.30	N14 (12.2)	2.30	1.44
5-5 130–136 cm	201.80	N14 (12.5)	2.31	1.36
6-2 130–136 cm	206.80	N12/N13 (12.9)	2.30	0.97
6-5 121–127 cm	211.21	N12/N13 (13.2)	1.97	1.06
7-2 131–136 cm	216.31	— (13.6)	2.25	1.15
7-5 110–116 cm	220.60	— (13.9)	2.18	1.10
8-2 114–120 cm	225.64	— (14.2)	2.33	1.12
8-5 114–120 cm	230.14	— (14.5)	2.20	1.18
9-2 114–120 cm	235.14	N10/N11 (14.9)	2.02	1.73
9-5 114–120 cm	239.64	N9 (15.2)	1.34	1.32
10-5 110–116 cm	249.10	N8 (15.9)	1.28	1.43
11-1 110–116 cm	252.60	N8 (16.1)	1.28	2.01
11-4 112–118 cm	257.12	N8 (16.5)	1.46	1.33
12-2 110–116 cm	263.60	N7 (17.0)	1.46	1.56
12-5 124–130 cm	268.24	N7 (17.4)	1.70	1.17
13-2 124–130 cm	273.24	N6 (18.2)	1.37	0.71
13-5 124–130 cm	277.74	N5 (19.2)	1.72	0.80
			1.58	1.01
14-2 129–135 cm	282.79	N4 (20.3)	1.78	1.00
15-2 117–123 cm	292.17	N4 (22.4)	1.50	1.62
			1.76	1.42
16-2 117–123 cm	301.67	P22 (24.5)	2.11	1.60
16-5 117–123 cm	306.14	P22 (25.5)	1.31	1.05
17-2 117–123 cm	311.17	P21b/P22 (26.7)	1.70	1.34
17-5 24–30 cm	314.74	P21b/P22 (27.5)	1.36	0.62
18-5 23–29 cm	324.23	P21b (29.6)	1.37	0.43
19-1 12–18 cm	327.62	P21a (30.3)	1.64	0.45
19-3 12–18 cm	330.62	P21a (30.7)	1.41	0.54
20-2 41–47 cm	338.91	P21a (32.0)	1.46	0.93
20-4 20–26 cm	341.70	P19/20 (32.4)	1.65	0.70
20-7 4–10 cm	347.04	P19/20 (33.2)	1.61	0.92
21-1 110–116 cm	347.60	P19/20 (33.3)	1.81	1.29
21-4 31–37 cm	351.31	P19/20 (33.8)	1.77	1.40
21-6 22–28 cm	354.22	P18 (34.3)	2.04	1.54
22-1 110–116 cm	356.60	P18 (34.6)	2.49	1.50
22-3 110–116 cm	359.60	P18 (35.1)	1.73	1.19

* Age estimated by interpolation of sedimentation rates between datums: FA *G. neponthes*, 204.3 m, 12.7 Myr BP; FA *Praeorbulina*, 255.0 m, 16.3 Myr BP; LA *C. dissimilis*, 270.5 m, 17.6 Myr BP; FA *G. dehiscentis*, 296.0 m s.b., 23.2 Myr BP; LA *Chiloguembelina*, 325.9 m, 30.0 Myr BP; LA *Pseudohastigerina*, 352.5 m, 34.0 Myr BP. In samples with replicates, mean values were plotted on Figs 1, 2.

The benthic foraminiferal carbon isotopic record at Site 563 shows three cycles of enrichments and depletions (Fig. 1). Enriched intervals occur in the Lower Oligocene (348–360 m s.b.), uppermost Oligocene to lowermost Miocene (292–311 m s.b.), and lower Middle Miocene (235–264 m s.b.). Depleted intervals, indicated with stipples in Fig. 1, occur in the 'Middle'-Upper Oligocene (315–347 m s.b.), Lower Miocene (268–283 m s.b.), and Middle Miocene (207–230 m s.b.).

The $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ records at western North Atlantic Site 563 show a similar pattern as the Oligocene record at Site 119 in the Bay of Biscay⁸ (4,447 m present depth; >3 km palaeodepth). Enriched ^{13}C values occurred in the early Oligocene at both sites and depleted values occurred in the late Oligocene. This carbon isotopic pattern may also be seen at Pacific Site 574 (K.G.M. and E. Thomas, in preparation), South Atlantic Site 522 (ref. 26) and Leg 74 sites²⁷. The Miocene pattern of enrichments and depletions is also observed at Sites 289 and 77 in the Pacific (2,206 m and 4,291 m present depth, respectively^{10,11}).

Comparison of Atlantic Site 563 and Pacific isotopic records (all data derived from analyses of *Cibicides* and *Planulina*, Fig. 2) shows that although similar $\delta^{13}\text{C}$ patterns are observed at all three locations, the Pacific sites are depleted in ^{13}C relative to the Atlantic site throughout the latest Oligocene through Middle Miocene (12–26 Myr BP). Before the ^{18}O enrichment of the Middle Miocene, Atlantic $\delta^{18}\text{O}$ values are also enriched by ~0.25–0.50‰ over Pacific values. After the Middle Miocene ^{18}O enrichment, $\delta^{18}\text{O}$ values are similar at all three sites, although $\delta^{13}\text{C}$ values remain depleted in the Pacific throughout the interval examined here.

The Middle Miocene $\delta^{18}\text{O}$ increase noted here has generally been attributed to the development of the Antarctic ice cap,

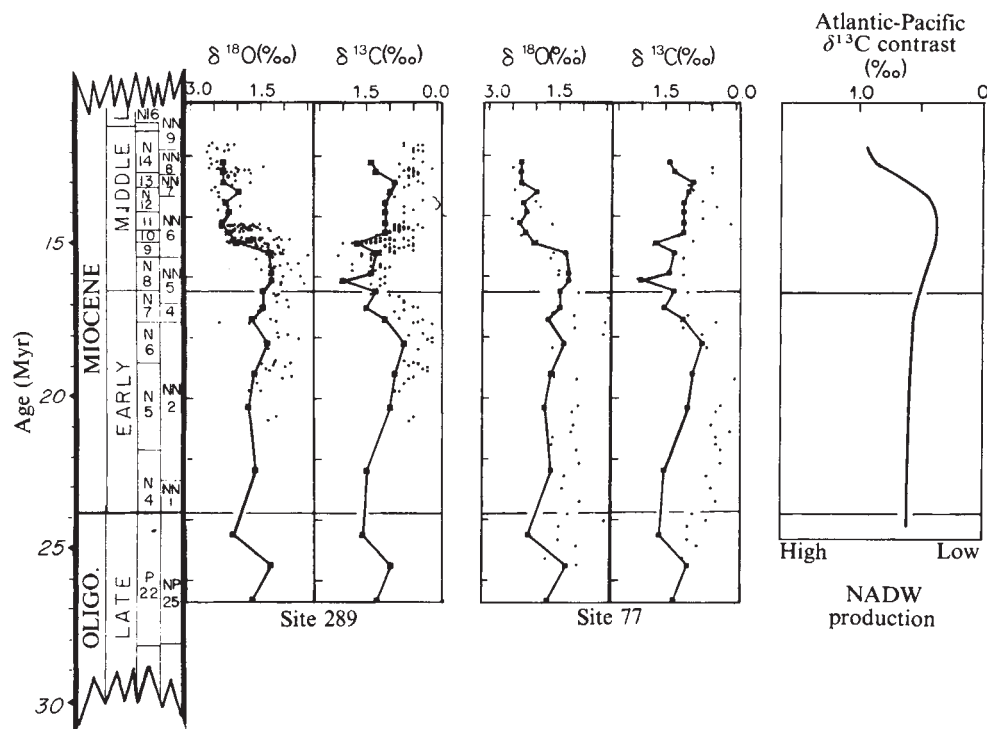


Fig. 2 Comparison of isotopic composition at Atlantic Site 563 (solid line with squares) and Pacific Sites 289 and 77 (points). Note that the Pacific $\delta^{13}\text{C}$ data (points) are depleted relative to the Atlantic data (squares). All isotopic data from analyses of *Cibicidoides* and *Planulina*; data from Sites 289 and 77 after ref. 11. Contrast obtained by fitting a hand-drawn curve through mean difference of Atlantic and Pacific data for the time intervals 12.2–12.4, 12.5–14.5, 14.5–17.0, 17.0–20.3, and 22.3–26.6 Myr BP. Ages obtained by interpolating between following datums using the time scale of Berggren *et al.*²⁰: Site 563, Table 1; Site 289, FA *G. nepenthes*, 348 m subbottom, FA *Praeorbulina*, 510 m s.b., FA *G. dehiscentis*, 654 m s.b. (datums after ref. 40); Site 77, FA *G. nepenthes*, 289 m s.b., FA *G. sicanus*, 17 m s.b., 16.6 Myr BP, FA *G. dehiscentis*, 289 m s.b. (datums after ref. 41).

while the latest Eocene–earliest Oligocene increase has been ascribed to a major bottom-water temperature drop^{1–5,10,11}. In contrast, Matthews and Poore²⁸ argued that the inverse was true. If a $\delta^{18}\text{O}$ increase is due to increased ice volume, then it should be globally synchronous. The slight apparent offset in time between the Middle Miocene enrichment at Site 289 and Site 563 (Fig. 2) is well within the errors of the biostratigraphical age assignments. The enrichments may be considered synchronous between oceans, as was suggested for the latest Eocene–earliest Oligocene enrichment⁹. Thus the synchrony or diachrony of the $\delta^{18}\text{O}$ enrichment cannot apparently be used here to differentiate causal mechanisms for these two time intervals.

Constraints still may be placed on the interpretation of the Middle Miocene $\delta^{18}\text{O}$ enrichment at Site 563. If the Middle Miocene enrichment is ascribed to an increase in $\delta^{18}\text{O}$ of seawater of 0.5–0.6‰ (ref. 10) (equivalent to a sea-level drop of ~60 m, ref. 30), then the enrichment at Site 563 (~0.7‰) represented little or no bottom-water temperature drop. A 2–3 °C bottom-water temperature drop must have occurred in the Pacific given these assumptions (that is, the Pacific enrichment is larger^{10,11}). On the other hand, if the Middle Miocene enrichment is due to temperature alone, bottom-water temperatures dropped in the western North Atlantic by ~3 °C and in the Pacific by 5 °C.

The $\delta^{18}\text{O}$ values noted in the early Oligocene (values for Zones P18 and P19/20 = 1.6–2.5‰) are slightly lower than modern *Cibicidoides* from the western North Atlantic (~2.6‰, ref. 23). The effect of melting the modern ice sheets would result in a decrease of mean oceanic $\delta^{18}\text{O}$ by 0.9‰ (ref. 3). Therefore, the assumption of an essentially ice-free world before the Middle Miocene^{1–3,10,11} would require early Oligocene bottom waters at Site 563 that were as cold or colder than at present (2.4–2.6 °C modern potential temperature²⁹; early Oligocene bottom-water estimates of 1–2 °C). In fact, such cold early Oligocene bottom-water temperatures are probably not compatible with an ice-free world. Thus, we suggest that significant ice volume existed during the early Oligocene.

Comparisons of Atlantic and Pacific $\delta^{13}\text{C}$ records can be used to infer abyssal circulation. Based on the correlation of lower $\delta^{13}\text{C}$ values of Holocene benthic foraminifera with older bottom waters^{22,23} (higher apparent oxygen utilization, higher CO_2 , and lower pH, refs 31–33), Curry and Lohmann³⁴ used carbon isotopic comparisons to decipher Quaternary abyssal circulation

changes. Variations in $\delta^{13}\text{C}$ may be caused by: (1) global changes in the ^{13}C budget^{35,36}; (2) abyssal circulation changes^{31–33}; and (3) local changes in productivity^{36,37}. Global changes in $\delta^{13}\text{C}$ which are caused by mechanisms such as increased input ^{13}C depleted terrestrial organic matter to the oceans, possibly due to sea-level changes^{35,36}, must be synchronous within and between oceans. The close correlations of $\delta^{13}\text{C}$ cycles observed in the Miocene of Pacific and Atlantic sites (Fig. 2) suggest that these changes are due to global changes in oceanic $\delta^{13}\text{C}$. Similar Oligocene $\delta^{13}\text{C}$ cycles noted in the Atlantic and Pacific suggest that these also were due to global changes. Such widely-distributed $\delta^{13}\text{C}$ patterns will be useful in future time-stratigraphical correlations.

The Oligocene to Middle Miocene Atlantic–Pacific $\delta^{13}\text{C}$ differences are interpreted as resulting from Atlantic to Pacific abyssal circulation. At present, the North Atlantic is enriched in ^{13}C relative to the Pacific by 1.0‰; this results from the production of NADW which is enriched in ^{13}C (refs 31–33). By analogy, we suggest that the western North Atlantic was enriched in ^{13}C relative to the Pacific in the Oligocene to Middle Miocene due to the production of young (high O_2 , low CO_2 ; high $\delta^{13}\text{C}$, refs 31–33) bottom waters analogous to NADW.

The Atlantic–Pacific $\delta^{13}\text{C}$ contrast was high in the latest Oligocene to early Miocene (0.7‰ average difference between 17 and 27 Myr BP) (Fig. 2). This contrast apparently decreased in the Middle Miocene (0.3–0.4‰ average differences between Site 563 and Pacific Sites 289 and 77, respectively, 15–17 Myr BP). The records subsequently diverged again in the later Middle Miocene. We interpret this as reduced production of bottom water analogous to NADW in the early Middle Miocene and subsequent increased production in the later Middle Miocene (Fig. 2).

The later Middle Miocene difference can be observed at other North Atlantic locations. Typical $\delta^{13}\text{C}$ values for *Cibicidoides* from the later Middle Miocene at Sites 116 and 366 are 1.2‰ (ref. 13) versus 0.6‰ for Pacific sites^{10,11}. Blanc *et al.*¹² and Blanc and Duplessy¹³ noted this offset of later Middle Miocene Pacific and Atlantic benthic foraminiferal $\delta^{13}\text{C}$ records, and suggested that the formation of bottom water analogous to modern NADW began near 12 Myr BP. However, in the early and Middle Miocene, Atlantic both Sites 563 and 366 are enriched in ^{13}C relative to the Pacific, although Site 116 yields values similar to Pacific values^{12,13}. Given the shallow location of Site 116 (1 km present and Oligo–Miocene palaeodepth; ref.

38) and its modern association with a region of high productivity³⁹, we suggest that the early Miocene depleted ^{13}C values at Site 116 represent local $\delta^{13}\text{C}$ variations in intermediate water, and that a significant $\delta^{13}\text{C}$ difference existed between the Atlantic and Pacific bottom water before 12 Myr BP. Thus our interpretation extends the production of bottom water analogous to NADW back until at least the Oligocene (older than 26 Myr BP).

Our interpretations of the $\delta^{13}\text{C}$ records are consistent with other monitors of abyssal circulation. Based on seismic stratigraphical studies, Miller and Tucholke¹⁵ suggested that northern sources for vigorously circulating bottom water (analogous to NADW) began near the end of the Eocene. They suggested that strong erosional conditions, together with the development of current-controlled sedimentation, indicated that such northern sources continued to influence the geological record throughout the Oligocene and early Miocene. However, a change to more widespread depositional conditions occurred in the latest early to Middle Miocene in the North Atlantic. They interpreted this as a reduction in intensity of abyssal circulation in the North Atlantic. The ^{13}C comparisons made here are consistent with this abyssal circulation model. The change to more depositional conditions near the early/Middle Miocene boundary appears to coincide with a convergence of Atlantic and Pacific $\delta^{13}\text{C}$ records which may be related to reduced production of NADW-like bottom water. Studies of Pacific Oligocene records are needed to establish whether the difference between Atlantic and Pacific carbon isotopic records began near the end of the Eocene, as predicted by the abyssal circulation model of Miller and Tucholke¹⁵.

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- Douglas, R. G. & Savin, S. M. *Init. Rep. DSDP Leg 17*, 591-605 (1973).
- Savin, S. M., Douglas, R. G. & Stehli, F. G. *Bull. geol. Soc. Am.* **86**, 1499-1510 (1975).
- Shackleton, N. J. & Kennett, J. P. *Init. Rep. DSDP Leg 29*, 743-755 (1975).
- Kennett, J. P. & Shackleton, N. J. *Nature* **260**, 513-515 (1976).
- Savin, S. M. *A. Rev. Earth planet. Sci.* **5**, 319-355 (1977).
- Keigwin, L. D. *Nature* **287**, 722-725 (1980).
- Boersma, A. & Shackleton, N. J. *Init. Rep. DSDP Leg 39*, 911-924 (1979).
- Miller, K. G. & Curry, W. B. *Nature* **296**, 347-352 (1982).
- Miller, K. G., Curry, W. B. & Ostermann, D. R. *Init. Rep. DSDP Leg 80* (in the press).
- Woodruff, F., Savin, S. M. & Doubbas, R. G. *Science* **212**, 665-668 (1981).
- Savin, S. M. *et al. Mar. Micropaleont.* **6**, 423-450 (1981).
- Blanc, P.-L. *et al. Nature* **283**, 553-555 (1980).
- Blanc, P.-L. & Duplessy, J.-C. *Deep-Sea Res.* **29A**, 1391-1414 (1982).
- Moore, T. C. *et al. Micropaleontology* **24**, 113-138 (1978).
- Miller, K. G. & Tucholke, B. E. *NATO Conf. Ser. Vol. 4*, 549-589 (Plenum, New York, 1983).
- Reid, J. L. & Lynn, R. J. *Deep-Sea Res.* **18**, 1063-1088 (1971).
- Worthington, L. V. *Johns Hopkins Oceanogr. Stud.* **6** (1976).
- Reid, J. L. *Deep-Sea Res.* **26**, 1199-1223 (1980).
- Berggren, W. A., Kent, D. & Flynn, J. *Geol. Soc. Lond. Spec. Pap.* (in the press).
- Berggren, W. A., Kent, D. & van Couvering, J. *Geol. Soc. Lond. Spec. Pap.* (in the press).
- Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39-55 (1973).
- Belanger, P. E., Curry, W. B. & Matthews, R. K. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **33**, 205-220 (1981).
- Graham, D. *et al. Mar. Micropaleont.* **6**, 483-497 (1981).
- Woodruff, F., Savin, S. M. & Douglas, R. G. *Mar. Micropaleont.* **5**, 3-13 (1980).
- Duplessy, J.-C., Moyes, J. & Pujol, C. *Nature* **286**, 479-482 (1980).
- Poore, R. Z. & Matthews, R. K. *Init. Rep. DSDP Leg 73* (in the press).
- Shackleton, N. J., Hall, M. A. & Boersma, A. *Init. Rep. DSDP Leg 74* (in the press).
- Matthews, R. K. & Poore, R. Z. *Geology* **8**, 501-504 (1980).
- Fuglister, F. C. *Woods Hole Oceanogr. Instr. Atlas Ser. 1* (1960).
- Fairbanks, R. G. & Matthews, R. K. *Quat. Res.* **10**, 181-196 (1978).
- Kroopnick, P. *Deep-Sea Res.* **21**, 211-217 (1974).
- Kroopnick, P. *Earth planet. Sci. Lett.* **49**, 469-484 (1980).
- Kroopnick, P., Weiss, R. F. & Craig, H. *Earth planet. Sci. Lett.* **16**, 103-110 (1972).
- Curry, W. B. & Lohmann, G. P. *Quat. Res.* **18**, 218-235 (1982).
- Shackleton, N. J. in *Fate of Fossil Fuel CO₂ in the Oceans* 401-427 (Plenum, New York, 1977).
- Broecker, W. *Geochim. cosmochim. Acta* **46**, 1689-1705 (1982).
- Berger, W. H., Diester-Haas, L. & Killingley, J. S. *Oceanol. Acta* **1**, 3-7 (1978).
- Berggren, W. A. *Init. Rep. DSDP Leg 12*, 965-1001 (1972).
- Milliman, J. D. *Deep-Sea Res.* **27A**, 959-963 (1980).
- Srinivasan, M. S. & Kennett, J. P. *Mar. Micropaleont.* **6**, 535-552 (1981).
- Keller, G. *Mar. Micropaleont.* **6**, 535-552 (1981).

Lesser Antilles isotopic evidence of the role of subducted sediment in island arc magma genesis

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Sediment recycling is an important factor in many current models of global geochemical evolution¹⁻⁴. Identification of a sedimentary component in the products of island arc magmatism is therefore of interest in evaluating such models. New isotopic data presented here for Martinique, in the Lesser Antilles island arc, span an exceptionally wide range of ratios which is consistent with a large contribution from continentally derived sediments. This contamination has produced the highest $^{87}\text{Sr}/^{86}\text{Sr}$, $^{206}\text{Pb}/^{204}\text{Pb}$ and lowest $^{143}\text{Nd}/^{144}\text{Nd}$ reported from an intra-oceanic arc. Data from other islands of the Lesser Antilles suggest that the incorporation of subducted sedimentary material is variable in extent along the arc, reaching a maximum in the vicinity of Martinique.

The geology of the Lesser Antilles volcanic arc, and tectonics of the Caribbean region are well documented⁵⁻⁸. The island of Martinique consists of several separate volcanic centres varying in age from 0 to 25 Myr (ref. 9). Following an extensive geochemical survey of the arc, Brown *et al.*¹⁰ concluded that the volcanics of Martinique are broadly calc-alkaline. However, more detailed studies indicate marked geochemical differences between the products of individual volcanic centres¹¹⁻¹³, and this is confirmed by the present work.

Sr, Nd and Pb isotopic analyses for Martinique, presented in Table 1, suggest that the volcanic products of each centre are derived from an isotopically homogeneous source. Samples from each volcano may be related by processes of fractional crystallization involving amphibole and plagioclase, probably occurring at shallow levels within the crust¹⁴⁻¹⁶. Major and trace element data, and rare earth element (REE) patterns, are consistent with this. The main alternative to be considered in modelling such a large range of isotopic ratios is that alteration of the samples with consequent elemental redistribution has occurred. This is thought unlikely because the samples analysed are petrographically fresh and show good correlations between radiogenic isotopic ratios and between other geochemical parameters. Significant differences between the source regions for each volcano on Martinique therefore seem to exist.

The data plotted on the Sr-Nd isotope correlation diagram (Fig. 1) describe a linear array, in part lying within the mantle array. Isotopic ratios extend from values comparable with those reported from many ocean islands^{17,18} ($^{87}\text{Sr}/^{86}\text{Sr}=0.704$, $^{143}\text{Nd}/^{144}\text{Nd}=0.5128$), to extremely radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ ($=0.709$) and unradiogenic $^{143}\text{Nd}/^{144}\text{Nd}$ ($=0.5121$) ratios. I believe these values represent the greatest deviation from oceanic mantle isotopic ratios reported for an island arc which is unequivocally developed on oceanic lithosphere⁵. Fewer Pb isotopic analyses are available, but $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ (Fig. 2a,b) show well correlated data sets extending over large ranges. Figure 2c shows a very good correlation between $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ resulting in a strongly curved hyperbola.

Several isotopically distinct contributions to parental arc magmas may be available in the source regions for each volcano¹⁹. Correlation between $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ indicates that high level crustal contamination is unlikely; fractionation of plagioclase, an abundant phenocryst phase in all samples, would variably alter Sr/Pb ratios in the liquids to generate trajectories differing from the simple hyperbola described in Fig. 2c. Contamination must therefore have occurred before plagioclase fractionation.

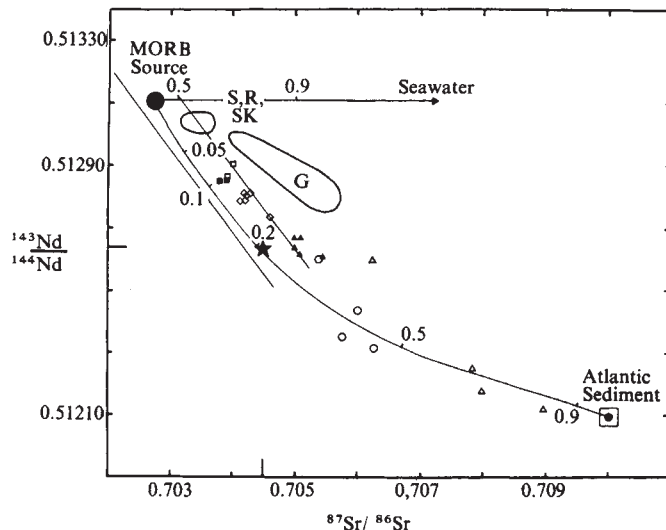


Fig. 1 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{143}\text{Nd}/^{144}\text{Nd}$ for Lesser Antilles island arc magmas. Individual data points are for Martinique samples, divided into volcanic centres as shown. Mixing lines are shown for comparative purposes between MORB and seawater, and between MORB and Atlantic sediment. The outlined field for Redonda, St Kitts and Statia represents 27 new paired analyses. Grenada data from Hawkesworth *et al.*³³. St Kitts samples were provided by Professor P. E. Baker. $\diamond$, Mt Pelée; $\square$, Piton Mt Conil; $\blacksquare$, Diamant; $\circ$, Pitons du Carbet; $\blacktriangle$, Morne Jacob; $\triangle$, Older Centres. S, Statia; SK, St Kitts; R, Redonda; G, Grenada.

If the isotopic correlations result from inhomogeneities within the mantle wedge, gross heterogeneity on a scale of kilometres is implied. Consequently age significance may be attached to the slope of $^{207}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ data (Fig. 2a). The calculated $^{207}\text{Pb}/^{206}\text{Pb}$ age is slightly greater than 3,000 Myr. This is unlikely to correspond to the dating of a mantle event because the lithosphere was involved in the extraction of oceanic crust less than 200 Myr ago. Mixing in the sub-oceanic asthenosphere probably precludes long term preservation of chemical heterogeneities. Source heterogeneity as the only mechanism for generating the observed data is therefore improbable.

The isotopic data may then reflect a mixing process, in the simplest case between two endmembers. The compositions of such endmembers can be constrained by the shapes and ranges of the arrays plotted in Figs 1 and 2. The mantle derived endmember is probably characterized by isotopic ratios similar to those reported elsewhere for mantle derived samples^{17,18,20}. Back projection of the data on all four diagrams (Figs 1, 2) results in intersection with the field of values for mid-ocean ridge basalts (MORB)^{21,22}, in particular average Atlantic MORB. It is not possible to distinguish isotopically between MORB source constituting the mantle wedge and unaltered ocean floor basalts of the subducted oceanic lithosphere. Although andesitic compositions have been obtained experimentally as minimum temperature melts of ocean floor basalt in its high-pressure form as quartz eclogite²³, such siliceous magmas are unlikely to rise far without reacting with the overlying ultrabasic mantle after separation at the Benioff Zone. Major and trace element variations together with field associations, indicate that andesitic lavas are most probably fractionates from basalts. Thus it is suggested that both andesites and basalts are related to a common parental magma derived from a sub-arc mantle with affinities to MORB source, which has been isotopically contaminated.

The characteristics of the contaminant include high $^{87}\text{Sr}/^{86}\text{Sr}$, high $^{206,207,208}\text{Pb}/^{204}\text{Pb}$ and low $^{143}\text{Nd}/^{144}\text{Nd}$. Such a slab-derived component is envisaged as being introduced metasomatically into the mantle wedge. The different isotopic ratios from each volcanic centre may then reflect variable proportions of the contaminant in the mantle source regions.

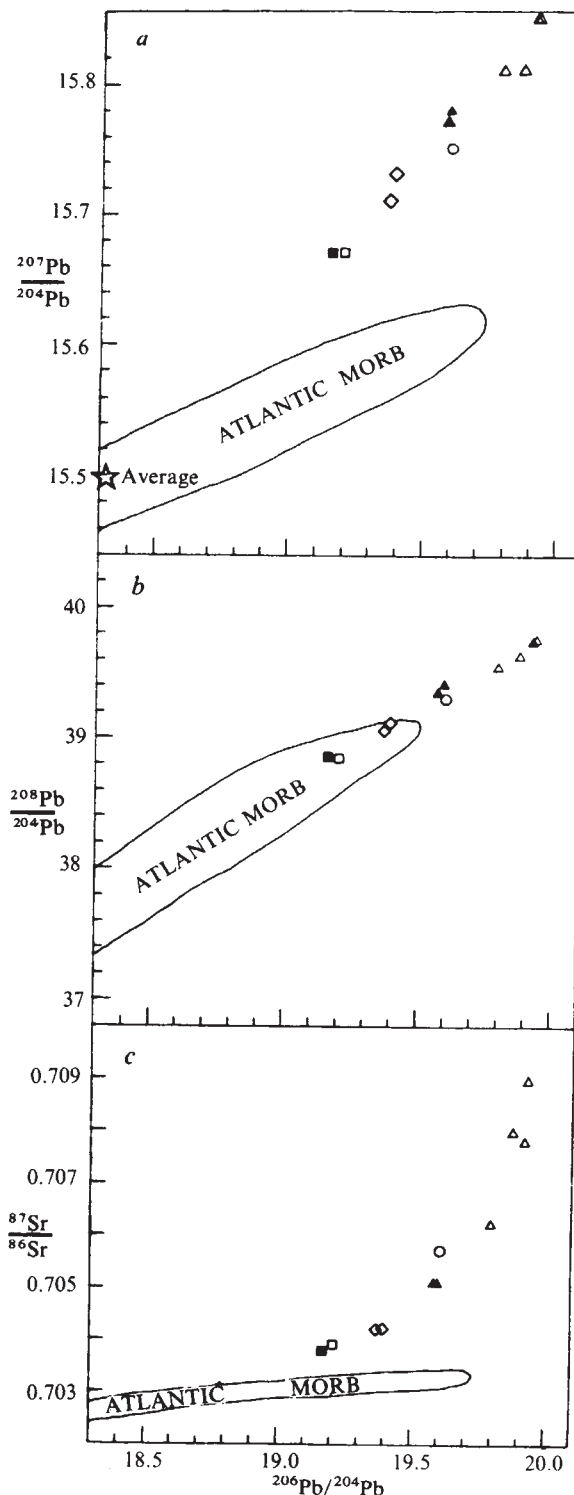


Fig. 2 $^{206}\text{Pb}/^{204}\text{Pb}$ versus a, $^{207}\text{Pb}/^{206}\text{Pb}$; b, $^{208}\text{Pb}/^{204}\text{Pb}$; c, $^{87}\text{Sr}/^{86}\text{Sr}$ for Martinique samples. Symbols as in Fig. 1.

For some island arc samples, such as those from the Marianas²⁴, minor translations to higher $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are thought to reflect the addition of a seawater derived fluid to the lherzolite of the mantle wedge. A mixing trajectory generated by addition of seawater to a MORB-like source is shown in Fig. 1. This is effectively a straight line forming part of a strongly upward convex hyperbola resulting from the very low concentration of Nd relative to Sr in seawater^{25,26}. The Martinique data, for which large displacements to unradiogenic $^{143}\text{Nd}/^{144}\text{Nd}$ accompany the increase in radiogenic Sr, clearly requires that the contaminating component possess a much lower Sr/Nd ratio than that of seawater. A mixing line is shown for comparative purposes between MORB and an Atlantic sediment composition

Table 1 Isotopic data for Martinique samples

Centre	Sample	SiO ₂ (%)	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Sr/ ⁸⁶ Sr	¹⁴³ Nd/ ¹⁴⁴ Nd	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb
Mt Pelée	M8215	60.7	25	286	0.70419 ± 2	0.512790 ± 14	19.395	15.73	39.11
	M8217	58.9	25	284	0.70414 ± 2	0.512793 ± 10	—	—	—
	M8220	57.8	21	279	0.70419 ± 3	0.512815 ± 7	19.374	15.71	39.05
	M8221	58.1	21	281	0.70426 ± 2	0.512812 ± 18	—	—	—
	M8222	59.1	25	274	0.70460 ± 6	0.512740 ± 21	—	—	—
	M8226	62.1	26	284	0.70423 ± 2	0.512810 ± 14	—	—	—
Piton Mt Conil	M8254	60.0	30	293	0.70391 ± 2	0.512867 ± 40	19.205	15.67	38.85
	M8264	57.5	19	295	0.70401 ± 2	0.512913 ± 15	—	—	—
Diamant	M8271	56.4	13	399	0.70376 ± 5	0.512850 ± 13	19.167	15.67	38.86
	21906	58.7	15	429	0.70389 ± 3	0.512857 ± 10	—	—	—
Pitons du Carbet	M8267	60.8	16	296	0.70611 ± 3	0.512438 ± 12	—	—	—
	M8268	62.9	57	256	0.70573 ± 2	0.512351 ± 10	19.604	15.75	39.31
	M8269	62.3	47	284	0.70625 ± 6	0.512319 ± 11	—	—	—
	21898	60.4	54	240	0.70535 ± 4	0.512603 ± 20	—	—	—
Morne Jacob	M8242	60.6	73	223	0.70544 ± 6	0.512607 ± 10	—	—	—
	M8243	59.6	64	217	0.70500 ± 6	0.512669 ± 16	—	—	—
	M8244	60.0	61	247	0.70508 ± 2	0.512667 ± 9	19.584	15.77	39.34
	M8270	59.4	62	244	0.70508 ± 3	0.512617 ± 13	19.587	15.78	39.41
	0303	60.1	67	215	0.70500 ± 8	0.512644 ± 8	—	—	—
Older centres	M8245	57.8	58	223	0.70798 ± 4	0.512178 ± 8	19.879	15.81	39.61
	M8272	61.4	138	178	0.70782 ± 2	0.512253 ± 9	19.926	15.85	39.74
	21919	58.2	58	212	0.70897 ± 3	0.512120 ± 18	19.929	15.85	39.75
	21910	56.0	92	191	0.70622 ± 5	0.512599 ± 15	19.795	15.81	39.55

Rb, Sr and SiO₂ concentrations determined by X-ray fluorescence. ⁸⁷Sr/⁸⁶Sr normalized to ⁸⁶Sr/⁸⁸Sr = 0.1194, ¹⁴³Nd/¹⁴⁴Nd normalized to ¹⁴⁶Nd/¹⁴⁴Nd = 0.7219. Errors are ± 2 s.e.m. Small age corrections may be necessary for M8272 and 21910, but these will be < 0.0004 on ⁸⁷Sr/⁸⁶Sr and do not significantly alter the plots. Similarly small corrections may be applicable to Pb isotopic ratios. Pb isotopic fractionation was monitored by frequent measurements of standard reference measurement SRM 891, which resulted in a mean value ²⁰⁶Pb/²⁰⁴Pb = 16.912 ± 0.026. Data have been subsequently normalized to SRM 981 = 16.937, to correct as far as possible for fractionation.

estimated by Luff²⁷. However, as bulk mixing is most unlikely, this curve simply illustrates the consequences of a lower Sr/Nd ratio in the contaminant. The actual contamination process will obviously be complex, involving partial melts or equilibrated fluids for which compositions cannot readily be constrained.

The correlation shown in Fig. 1 can be generated by simple mixing between a contaminant and a mantle endmember with similar Sr/Nd ratios. The trend of Pb isotopic ratios is to highly radiogenic compositions (Fig. 2a, b) characteristic of terrigenous cratonic detritus rather than pelagic sediments²⁸. The curvature of the data in Fig. 2c indicates that the Sr/Pb ratio of the contaminant is less than that of the mantle endmember, and whilst back projection of the least radiogenic samples is towards MORB values, asymptotic behaviour at the most radiogenic compositions limits ²⁰⁶Pb/²⁰⁴Pb to ~20.0. This is significantly higher than ²⁰⁶Pb/²⁰⁴Pb ratios reported for Pacific sediment by Sun²⁹. The slab-derived endmember of the mixing relations displayed by the Martinique data may therefore be characterized by the following estimated isotopic parameters; ¹⁴³Nd/¹⁴⁴Nd < 0.5120, ⁸⁷Sr/⁸⁶Sr > 0.710 and ²⁰⁶Pb/²⁰⁴Pb ≈ 20.0. It is not possible to constrain the contaminant fully, but analyses in progress on sediments cored to the East of the Barbados accretionary prism during DSDP Leg 78³⁰ may provide suitable mixing endmembers.

Isotopic data from Martinique contrast markedly with that from other islands of the Lesser Antilles arc. New data for the northern islands of St Kitts, Statia and Redonda, which are in agreement with previously published analyses^{6,31,32}, are plotted on Fig. 1. Tight clustering close to MORB values suggests predominance of a MORB type source in magma generation. Slight displacement from the mantle array may indicate minor contamination, but the data are too restricted in range to suggest any specific type of contamination. Isotopic data from the southern islands, particularly Grenada³³, whilst showing a substantial range, do not extend to the extreme values present from Martinique. Alkaline lavas from Grenada, if generated from a mantle source alone^{34,35}, would require this to be quite strongly heterogeneous to evolve the reported isotopic compositions. Hawkesworth *et al.* conclude that radiogenic Sr has been added to the mantle source of Grenada samples through subduction³³.

The isotopic data reported here from Martinique argue strongly for significant contamination of the erupted magmas by a subducted terrigenous sediment. Data available from other islands along the Lesser Antilles arc indicate that ranges of Sr, Nd and Pb isotopic ratios are more restricted. This is thought to reflect lateral variations along the arc in the proportions of different slab derived contributions to the island arc magmas.

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- Allègre, C. J. *Tectonophysics* **81**, 109–132 (1982).
- Cohen, R. S. & O'Nions, R. K. *Earth planet. Sci. Lett.* **61**, 73–84 (1982).
- O'Nions, R. K., Evensen, N. M. & Hamilton, P. J. *Phil. Trans. R. Soc. A* **297**, 479–493 (1980).
- Hofmann, A. W. & White, W. M. *Yb. Carnegie Instn Wash.* **79**, 477–483 (1980).
- Westbrook, G. K. *Geophys. J. R. astr. Soc.* **43**, 201–242 (1975).
- Donnelly, T. W., Rogers, J. J., Pushkar, P. & Armstrong, R. L. *Geol. Soc. Am. Mem. No.* **130**, 181–224 (1971).
- Tomblin, J. F. in *Trans Caribbean Geological Conf.* VI, Isla de Margarita 277–282 (Impreso por Cromotip, Caracas, 1972).
- Rea, W. J. in *Andesites* (ed. Thorpe, R. S.) 167–185 (Wiley, New York, 1982).
- Briden, J. C., Rex, D. C., Faller, A. M. & Tomblin, J. F. *Phil. Trans. R. Soc. A* **291**, 485–528 (1979).
- Brown, G. M., Holland, J. G., Sigurdsson, H., Tomblin, J. F. & Arculus, R. J. *Geochim. cosmochim. Acta* **41**, 785–801 (1977).
- Westercamp, D. *Bull. Volcan.* **39**, 175–200 (1976).
- Westercamp, D. & Tazieff, H. in *Guides Géologiques Régionales; Martinique and Guadeloupe* (Masson, Paris, 1980).
- Gunn, B. M., Roobol, M. J. & Smith, A. L. *Bull. geol. Soc. Am.* **85**, 1023–1030 (1974).
- Arculus, R. J. & Wills, J. K. *J. Petrol.* **21**, 743–799 (1980).
- Powell, B. M. *Earth planet. Sci. Lett.* **39**, 162–172 (1978).
- Cawthorn, R. G. & O'Hara, M. J. *Am. J. Sci.* **276**, 309–329 (1976).
- White, W. M. & Hofmann, A. W. *Nature* **296**, 821–825 (1982).
- O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34**, 13–22 (1977).
- Wilson, M. & Davidson, J. P. *Phil. Trans. R. Soc.* (in the press).
- Zindler, A., Jagoutz, E. & Goldstein, S. *Nature* **298**, 519–523 (1982).
- Dupré, B. & Allègre, C. J. *Nature* **286**, 17–22 (1980).
- Cohen, R. S., Hamilton, P. J., Evensen, N. M. & O'Nions, R. K. *Nature* **283**, 149–153 (1980).
- Green, T. H. & Ringwood, A. E. *Earth planet. Sci. Lett.* **1**, 307–316 (1966).
- Meijer, A. *Bull. geol. Soc. Am.* **87**, 1358–1369 (1976).
- Hawkesworth, C. J. & Powell, B. M. *Earth planet. Sci. Lett.* **45**, 237–248 (1979).
- Hawkesworth, C. J. in *Andesites* (ed. Thorpe, R. S.) 549–571 (Wiley, New York, 1982).
- Luff, I. W. thesis, Univ. Leeds (1982).

28. Church, S. E. *Earth planet. Sci. Lett.* **29**, 175–188 (1976).
29. Sun, S. S. *Phil. Trans. R. Soc. A* **297**, 405–445 (1980).
30. Casey Moore, J. et al. *Bull. geol. Soc. Am.* **93**, 1065–1077 (1982).
31. Hedge, C. E. & Lewis, J. F. *Contr. Miner. Petrol.* **32**, 39–47 (1971).
32. Rea, W. J. *J. geol. Soc. Lond.* **130**, 341–366 (1974).
33. Hawkesworth, C. J., O'Nions, R. K. & Arculus, R. J. *Earth planet. Sci. Lett.* **45**, 237–248 (1979).
34. Arculus, R. J. *Bull. geol. Soc. Am.* **87**, 612–624 (1976).
35. Shimizu, N. & Arculus, R. J. *Contr. Miner. Petrol.* **50**, 231–240 (1975).

Origin of string patterns in boreal peatlands

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The detailed stratigraphical analyses supported by field observations on mires in Labrador and northern Minnesota reported here provide the basis for an explanation of the development of the surface pattern of alternating peat ridges (strings) and linear depressions or pools (flarks) in boreal fens. The developmental sequence involves: drainage impediment and the concomitant initiation of peat accumulation; development of linear patterns as a result of changing hydrological conditions and differential rates of peat accumulation of hummock and hollow plant communities; and subsequent enlargement and coalescence of pools through the degradation of the underlying peat and the intervening ridges. We show here that many of the processes may be active in the development of the pool patterns on raised bogs as well as on fens.

A pattern of strings and flarks in peatlands forms one of the most distinctive landforms in boreal forests, where they were first studied in Fennoscandia, Alaska, and the Hudson Bay Lowlands^{1–4}. American authors previously referred to these features as string bogs, although now it is more common to follow Scandinavian concepts that distinguish nutrient-rich fens from the nutrient-poor bogs that are fed only by atmospheric precipitation. In North America patterned fens reach their major southernmost occurrence in Minnesota^{5,6}, where the shallow flarks and weakly defined ridge patterns are indistinct on the ground but are easily discerned from the air. In more northerly and maritime locations such as Labrador, however, robust strings stand 15–20 cm above open-water pools, which may be more than 20 cm deep, creating ladderlike arrangements of successive pools and ridges resembling a gentle flight of terraces. The orientation of the linear patterns is invariably transverse to the direction of water flow, as controlled by the often imperceptible slope of the peat surface (Fig. 1).

The status of the problem is perhaps best summarized by Washburn⁷, who lists several hypotheses. Those that depend on rifting of peat during downslope slumping^{8–10} fail because of the extremely low slopes involved in most areas, the fibrous nature of the peat, and the absence of supporting stratigraphical evidence. Those that postulate a relation to permafrost processes^{11,12} fail because permafrost is now absent from large areas with string patterns in eastern North America and apparently never occurred in these regions during the time of peatland development, as inferred from climatic reconstructions of pollen diagrams^{13–15}. Differential heaving during annual frost may serve in some instances to accentuate the existing surface topography¹⁶ but has not been documented in the developmental process. The hypothesis that appeals to the accumulation of plant debris in transverse riffles during spring runoff^{16–18} likewise suffers from inadequate observations of the process, as does the proposal that some sedge or shrub species grow differentially across the slope to form ridges¹⁹. Arguments suggesting a relationship between surface patterning and substratum topography (ref. 18 and refs therein) are not supported by

stratigraphical evidence, although large pools have been shown to be associated with depressions in the mineral substratum on raised bogs in Scotland²⁰. Thus despite many decades of observation by peatland ecologists and geomorphologists, no consensus has emerged concerning the origin and development of the patterns, particularly the diagnostic linearity²¹.

We have carried out field studies of patterned fens in southern Labrador and adjacent Nouveau Quebec¹⁵ and in northern Minnesota²². The results are believed to be generally applicable to patterned fens throughout the boreal forest and may also apply to the development and particularly the enlargement of arcuate pool patterns on bogs.

The hypothesis has three elements, involving: (1) initial accumulation of peat; (2) development of linear patterns; and (3) subsequent enlargement of pools.

(1) Peat accumulation can be initiated when drainage is impeded at the base of a gentle slope. A special case involves the formation of an ice-push ridge at a lake shore. The trees that colonize such a ridge frequently serve as a snow fence against the winter winds that blow across the lake ice. Melting of the deep snow that accumulates to the lee of the fence may increase the waterlogged conditions at the base of the slope, and under a suitable climatic regime a small peatland may form. In time the peatland gradually extends upslope by continued drainage impediment. In northern Minnesota peat began to accumulate on poorly drained lake plains at the end of the mid-postglacial dry period.

(2) Accumulating peat tends to smooth out irregularities on the slope, thus converting channelled flow to sheet flow. The peat itself has low hydraulic conductivity, particularly when humified, and the surface water tends to pond on the surface in low spots. A microtopography develops as hummocks of *Carex exilis*, *Scirpus cespitosus* and *Sphagnum* grow vigorously beside hollows occupied by such weak peat producers as *Carex limosa* and *Menyanthes trifoliata*²³. During times of high water when the hollows are inundated, plant growth along the pool margins is inhibited. As the surface rises with continued peat accumulation, adjacent pools at the same level across the slope gradually join together and remain flooded as the intervening hummock area is inundated, whereas a pool positioned upslope from another pool may simply drain downslope. Linear pools thus formed can be irregularly extended along the contours of the peatland through the gradual merging of adjoining depressions^{24–26}. The explanation is equally suitable for the genesis of arcuate pools on raised bogs.

(3) Once a pool has standing water, vascular plant growth is reduced, the vegetation cover becomes sparse, and peat accumulation ceases. Degradational processes become increasingly active, and the peat on the pool floor and margins begins to break up. The process probably involves active microbial decomposition in an oxygenated environment^{27,28}, although various erosional processes may contribute to the degradation. Methanogenesis in the underlying peat is suggested by the escape of bubbles when the flocculent bottom is disturbed, and it may be important in the flotation of peat detritus that accumulates on the bare floor. This latter process further exposes the organic material to the oxygenated water column, thereby enhancing decomposition. Pools are thus deepened and widened by peat decomposition, and by the time the pool is 15–30 cm deep the entire floor may be bare of vegetation. As the pool sides are degraded, the intervening ridges between pools may be broken, and the pools thus coalesce preferentially along the contour. In some cases the ridges between pools are so high and are so firmly bound by shrub growth that they resist decomposition at the surface but become undermined at depth on both sides, and during times of flooding the water may siphon from one pool to the next downslope by developing a channel beneath the ridge, producing a crater in the pool bottom on each side of the ridge.

Support for this hypothesis comes from stratigraphical studies at Leech fen (53°10' N, 58°45' W) in southeastern Labrador¹⁵. The fen is located on the gentle slopes beside a shallow bay in

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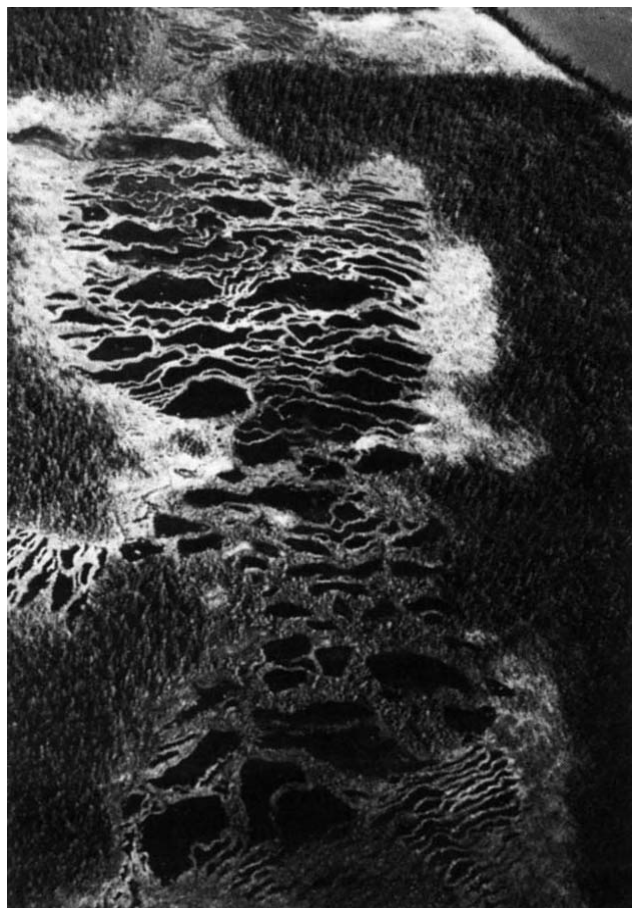


Fig. 1 Stepwise slope of a patterned fen in western Labrador exhibits closely-spaced ridges and narrow pools on steep slopes in the lower edge of the photo and anastomosing strings and broad pools on gentle slope in the centre. The fen slopes to the lake at the top of the photo where an ice-push ridge impedes drainage. Total length is ~500 m.

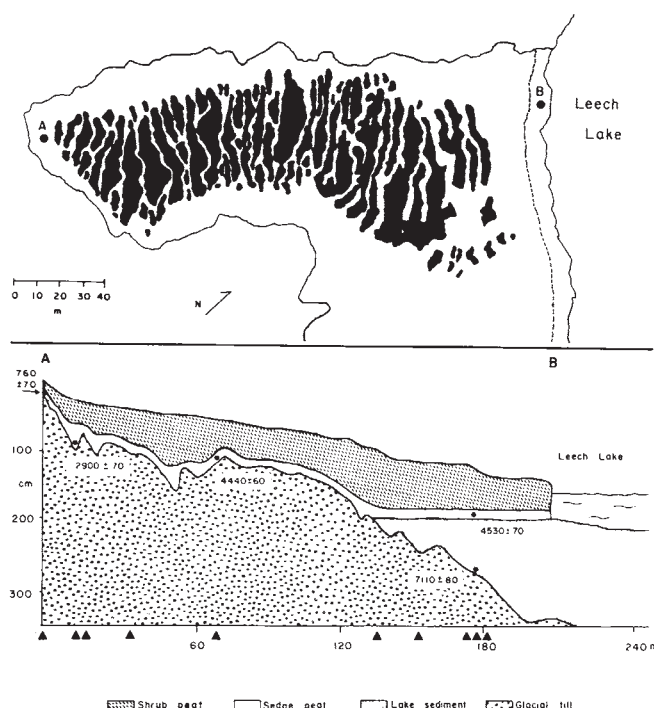


Fig. 2 Map and cross-section (along transect from points A to B) of Leech fen in southeastern Labrador. Progressively younger radiocarbon dates of basal sediments upslope indicate the gradual paludification of upland areas. Adapted from ref. 15.



Fig. 3 Disintegration and swamping of the intervening strings lead to the coalescence of flarks and formation of extensive pools on the gentle slopes of a fen in western Labrador.

a lake that is fringed by a low, forested ice-push ridge. A transect of peat cores up the length of the fen indicates that an unpatterned sedge fen developed upslope from the bay, while a sedge mat was simultaneously extending across the sediments in the shallow water (Fig. 2). Gradual paludification of the upland is demonstrated by progressively younger radiocarbon dates upslope: basal peat near the lake shore yielded a radiocarbon date of $4,400 \pm 60$ (WIS-1355) yr BP, two-thirds of the way upslope a date of $2,900 \pm 70$ (WIS-1356) yr BP, and at the upslope end of the fen 760 ± 70 (WIS-1403) yr BP.

The development of a hummock/hollow topography in a vegetation of *Sphagnum*, ericaceous shrubs, and sedges is indicated in the history of Leech fen by the pollen and macrofossil evidence from peat cores and is corroborated by numerous analogues in other portions of this large mire complex. Pool deepening by decomposition is suggested by the peat stratigraphy and by comparison of pollen diagrams for cores from a pool bottom and an adjacent ridge. Radiocarbon dates of firm peat on the pool floor below the loose mat of detritus are $1,210 \pm 70$ (WIS-1353) and 410 ± 70 yr BP (WIS-1404) for pools on the lower and mid-slope of the fen respectively, indicating either that no deposition has occurred in several hundred years or that the pool has deepened by decomposition. Active degradational processes are demonstrated by the absence of any accumulation of pool sediment, and by the fact that the loose detritus on top of the firm peat on the pool floor consists largely of a scatter of wood fragments, representing the refractory ligneous remains of shrub peat, from which the moss and sedge components have been lost by decomposition. Lundqvist in Sweden²⁷ long ago attributed to decomposition the upward truncation of a pollen

sequence from a pool, but the idea was never integrated into an explanation for the patterns.

Field observations of patterned fens and raised bogs elsewhere in southern Labrador and adjacent eastern Quebec indicate abundant evidence for pool enlargement and deepening by peat degradation. The largest pools (on both fens and bogs) have lost most of their peat by this process, so that the floor is the mineral substratum, perhaps with patches of peat detritus. It is probable that many of the large shallow ponds on the extensive wetlands on the flat plateau of southeastern Labrador were formed by degradation of a once more continuous peatland (Fig. 3). Conditions favourable for the aerobic decomposition of peat in pools were demonstrated in a patterned fen in western Labrador (Shovel fen, $52^{\circ}41' N$, $65^{\circ}55' W$), where the dissolved oxygen content was $3.7\text{--}5.3 \text{ mg L}^{-1}$ in three small pools (100 m^2 in area and 7-cm deep) and $7.2\text{--}8.2 \text{ mg L}^{-1}$ in six pools that were larger and deeper.

Detailed inspection and interpretation of aerial photographs of extensive regions of patterned mires in eastern Canada support the proposed method of pool enlargement. In most

patterned mires, remnant hummocks occur in the middle of pools, and many ridges stretching only part way across a pool (Figs 1–3). These features result from the degradation and inundation of low points on ridges between pools, as the water table rises in the mire.

The developmental history of large peatlands may be highly complex and dynamic, for in some cases even a large pool may drain completely by decomposition of a downslope peat ridge, and the process of peat accumulation and pattern formation may start on the drained pool floor, with the formation of a *Carex limosa* lawn followed by the local hummock growth of *Scirpus cespitosus* and *Sphagnum*. The contrasting processes of

peat accumulation and peat decomposition develop in a systematic way that reflects a variety of environmental controls. The result is a display of landscape patterns that are perhaps the most consistently spectacular of any to be found in the boreal forest.

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1. Cajander, A. K. *Acta for. fenn.* **2**, 1–208 (1913).
2. Ruuhijärvi, R. *Ann. Bot. Vanamo* **30**, 1–360 (1960).
3. Drury, W. H. *Contr. Gray Herb. Harv.* **178**, 1–130 (1956).
4. Sjörs, H. *Nat. Mus. Can. Bull.*, *Contr. Bot.* **18b**, 45–133 (1963).
5. Heinselman, M. L. *Ecol. Monogr.* **33**, 327–372 (1963).
6. Glaser, P. H. *et al. J. Ecol.* **69**, 575–599 (1981).
7. Washburn, A. L. *Geocryology* (Edward Arnold, London, 1979).
8. Andersson, G. & Hesselman, H. *St. Skogsforsöksanstalt Medd.* **4**, 41–110 (1907).
9. Auer, V. *Acta for. fenn.* **12**, 23–145 (1920).
10. Pearson, M. C. *J. Ecol.* **48**, 647–666 (1960).
11. Hamelin, L. E. *Cah. geogr. Québ.* **12**, 87–106 (1957).
12. Schenk, E. *Int. Conf. Permafrost N.R.C. Can. Publ.* **1287**, 155–159 (1966).
13. Janssen, C. R. *Can. J. Bot.* **46**, 1397–1408 (1968).

14. Griffin, K. O. *Can. J. Bot.* **55**, 172–192 (1977).
15. Foster, D. R. & King, G. A. *J. Ecol.* (in the press).
16. Tantt, A. *Acta for. fenn.* **4**, 1–24 (1914).
17. Drury, W. H. *Contr. Gray Herb. Harv.* **178**, 1–130 (1956).
18. Sakaguchi, Y. *Bull. Dept. Geogr. Univ. Tokyo* **12**, 35–58 (1979).
19. Sjörs, H. *Arkiv. Bot.* **33**, 1–96 (1946).
20. Boatman, D. J. *et al. J. Ecol.* **69**, 897 (1981).
21. Moore, P. D. *Nature* **300**, 110 (1982).
22. Glaser, P. H. *et al. J. Ecol.* **69**, 575–599 (1981).
23. Glaser, P. H. & Wheeler, G. A. *Proc. 6th Int. Peat Congr.* 31–35 (1980).
24. Sjörs, H. *Arkiv. Bot.* **33**, 1 (1946).
25. Gorham, E. *Q. Rev. Biol.* **32**, 145–166 (1957).
26. Boatman, D. J. & Tomlinson, R. W. *J. Ecol.* **65**, 531–546 (1977).
27. Lundqvist, G. *Sver. geol. Unders. Forhandl. Ser. Ca21*, 1–213 (1951).
28. Sjörs, H. *Nat. Mus. Can. Bull.* **186**, 45–133 (1963).

Ramapithecines from China: evidence from tooth dimensions

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Data obtained from ramapithecine specimens found in Asia, Africa and Europe^{1–6} have suggested the existence of two major subgroups, *Ramapithecus* and *Sivapithecus*, with *Ramapithecus* having pre-human status^{7,8}. Recently, however, it has been proposed that the fossils all belong to a single group, *Sivapithecus*, which is more closely related to the apes, in particular the orang-utan^{9–13}. Here we analyse data from a series of similar fossils which have been found in late Miocene coalfields in Lufeng, Yunnan Province, China^{14–16}. These include a number of almost complete jaws and five partial skulls which are more complete than any others so far known^{17–21}. A statistical analysis of the overall dimensions of the large number of teeth included in these finds shows that the differences between the groups previously assigned to *Ramapithecus* and *Sivapithecus* are greater than those found between the sexes in the most sexually dimorphic of the living great apes. Within the groups the distribution is bimodal and we suggest each group contains sex subgroups.

The cranial fragments examined indicate that the skull of the group called *Ramapithecus* is smoother and more round and bulging than the crania of modern apes. The nuchal crest is not marked and the nuchal plane is smooth. The face is rather short and the foramen magnum situated in a much more forward position than in modern apes. The dental arch is divergent, with small vertically oriented front teeth. The canines are also small and project only slightly from the other teeth. Among the three molars the second is the biggest and all have thick enamel. The skull of the group named *Sivapithecus* contrasts considerably, having generally more ape-like configurations in each of the above features, and especially in possessing an orang-utan-like configuration of the brow ridges and concave facial profile

(although both specimens seem to have these characteristics to some degree).

These new finds in China also include several hundred teeth either *in situ* in portions of jaws, or as isolated teeth. The existence of these samples of teeth, larger indeed than the entire collection of such teeth outside China, now allow new investigations of these forms.

Although much is to be learned by further detailed study of such a large assemblage of teeth, it is also possible to learn much about the general characteristics of these forms through simple quantitative studies of the overall dimensions of the teeth.

Accordingly the dimensions (length and breadth) of these teeth have been measured (by W. R.) and analysed using simple statistical methods (by C. E. O.). From these studies it may be possible to understand something of the number of groups represented in the collection, their general variability and, possibly, their sexual dimorphism and socio-sexual structure.

The fossils were discovered in late Miocene aged coalfields of Lufeng (Shihuiba) in Yunnan Province. The different, but contiguous, areas of the site that were examined during each excavation, and the details of the local stratigraphy are given in the original publications^{22–24}.

The fossils were assigned to the genera *Sivapithecus* and *Ramapithecus* by Professor Wu on the basis of morphological examination and before the measurements were analysed. In particular these examinations were undertaken by comparing teeth (about half of the total number) that were found isolated with teeth found *in situ* in portions of upper and lower jaws, situations in which designations could be made on the basis of features of the jaws and skulls as well. Clearly, these attributions should be considered tentative because of changing views about ramapithecines; however, Professor Wu and his colleagues are extremely experienced in this general area, having examined and compared almost 1,000 ramapithecine teeth from this site alone.

The teeth are represented by every position in both upper and lower jaws. The sizes of the samples at each tooth position range from as few as 17 for the lower first incisor to as many as 44 for the lower third molar and lower canine of *Ramapithecus*. Equivalent samples for *Sivapithecus* range from as few as 16 teeth for the lower second incisor to 49 for the lower second molar. Maximum length and breadth were taken on each of a total of 934 adult, unbroken and not too badly worn teeth shared approximately equally between the two genera. In addition, in order to increase the sizes of the samples as

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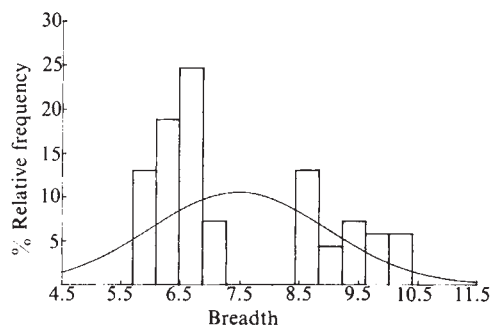


Fig. 1 Histogram of the breadth of the lower canine in all specimens of *Ramapithecus* and *Sivapithecus*. Two blocks of specimens are clearly located; each coincides absolutely with Professor Wu's morphological assessment; they are separated by a large interval; the entire histogram is significantly different from the fitted normal curve ($P < 1:1,000$). The difference in size of the two parts of the histogram results from the specimens being treated as a single group, as there are about twice as many specimens available for *Ramapithecus* as for *Sivapithecus* for this particular tooth.

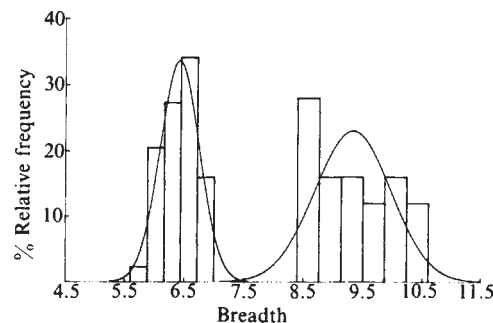


Fig. 2 Histograms of the breadth of the lower canine in *Ramapithecus* and *Sivapithecus* treated separately. The same two blocks of specimens are apparent as in Fig. 1, and because of the finer grade of the histogram cells, they can be seen to be separated by an even larger interval than in Fig. 1. Normal curves have been fitted to each histogram separately and even these scarcely overlap at all. In this case, however, they are not statistically significantly different from the overlain histograms ($P > 0.5$). Although the number of specimens is about twice as large for *Ramapithecus* as for *Sivapithecus*, as in Fig. 1, the two histograms are approximately the same size because they are treated separately (y axis = % relative frequency).

much as possible, a few additional lengths or breadths were included even when the condition of individual teeth prevented the other measurement from being included. Thus, the sample size for each dimension for each tooth is not always the same. Table 1 gives details of the full numbers of dimensions and teeth examined in each genus.

Because the number of individual measurements at each locus in both upper and lower jaws is generally between 25 and 35, we have chosen to study the distributions of these measurements using means, standard deviations, computed histograms and fitted normal curves. The significance of the differences between the actual forms of the histograms and the fitted normal curves have been estimated using a standard χ^2 test.

The first study compares all the specimens irrespective of designation by Professor Wu. The marked difference between *Ramapithecus*, which is always smaller, and *Sivapithecus* is clear, and is especially obvious in examination of individual histograms and fitted normal curves for individual measurements (Fig. 1).

The main study examines the two groups separately as assigned. The means and standard deviations for lengths and breadths for each tooth position within both upper and lower jaws for species groups are also given in Table 1. They demon-

strate differences between the two groups of specimens that are far greater than those found between the sexes in the most sexually dimorphic of the living great apes^{25,26}. There are very few dimensions for teeth where the differences between the groups are not statistically significant. There are only a few dimensions where the histograms overlap slightly. Most histograms are separated by a large interval (for example, Fig. 2) and, indeed, for many of the dimensions the separations between the two groups are so great that even the tails of the fitted normal curves scarcely overlap (Fig. 2).

Of perhaps even greater significance, however, is the fact that, for most dimensions of most teeth for both *Ramapithecus* and *Sivapithecus*, the histograms do not appear normal but bimodal (Fig. 3). This could mean that each of the groups comprises two subgroups. These could be species groups within genera, but considerable information as detailed below suggests that they are sex subgroups. Thus, the number of bimodal distributions along the tooth row that are statistically significantly different from normal is greater in *Sivapithecus* than in *Ramapithecus*, being, in *Sivapithecus*, of an order (16 statistically significant

Fig. 3 Histograms of the length of the upper second molar and the breadth of the lower third molar in *Ramapithecus* and *Sivapithecus* treated separately but plotted on the same scales. In these two examples there is slight overlap of the histograms, and slightly more overlap of the fitted normal curves for each dimension, although statistical tests show that the two groups are significantly different from one another for each dimension ($P < 0.01$). These particular dimensions do not provide the clearest separations between the two groups, but they are shown because they provide examples of the equal bimodal nature of the histograms in *Ramapithecus* and the unequal (smaller upper peak) bimodal character of the histograms in *Sivapithecus* occurring in the same dimension in both groups.

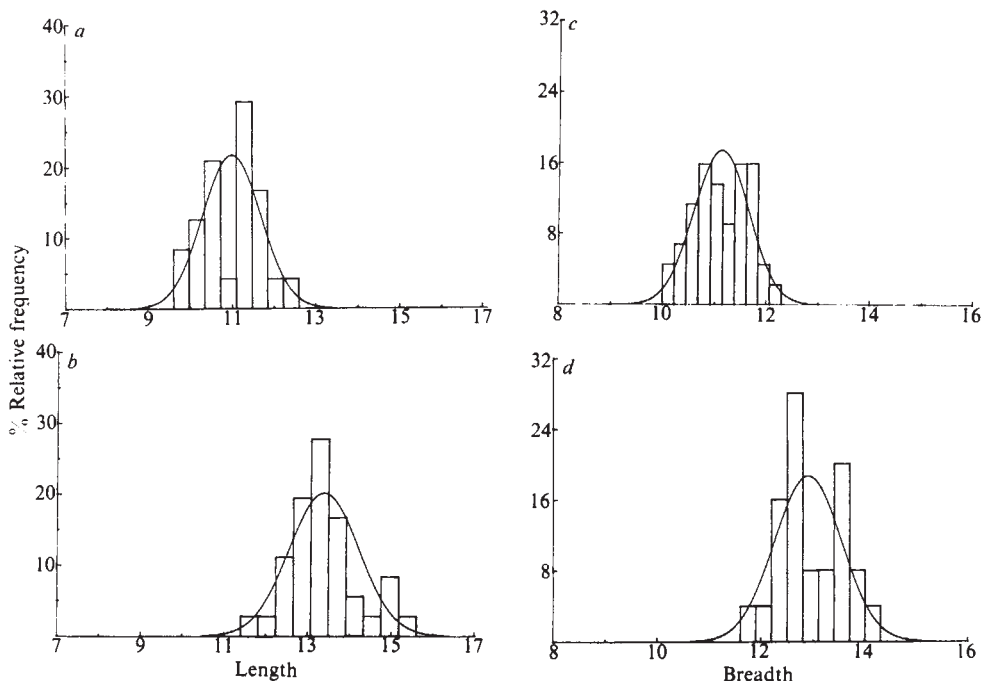


Table 1 Basic statistical data for tooth dimensions

<i>Ramapithecus</i>						<i>Sivapithecus</i>				
Tooth	<i>n</i>	$\bar{x}$ length	s.d. length	$\bar{x}$ breadth	s.d. breadth	<i>n</i>	$\bar{x}$ length	s.d. length	$\bar{x}$ breadth	s.d. breadth
I ¹	31	8.84	0.40	8.15	0.29	48/46	10.07	0.56	9.46	0.38
I ²	21	5.55	0.42	6.33	0.38	18	6.57	0.45	7.71	0.71
C'	27/24	10.25	0.48	9.15	0.33	19	14.64	1.19	11.57	0.81
P ³	24	7.50	0.46	10.39	0.60	28	12.41	0.62	12.41	0.69
P ⁴	28	7.00	0.43	10.97	0.61	34/31	9.46	0.61	12.80	0.47
M ¹	32	10.11	0.43	11.25	0.54	31/30	11.79	0.58	13.03	0.46
M ²	24	10.99	0.69	12.18	0.57	36	13.38	0.84	14.77	0.82
M ³	24	10.58	0.61	11.83	0.63	22/24	12.75	1.08	14.36	0.53
I ₁	17/20	5.09	0.21	6.78	0.35	24/22	5.86	0.34	8.35	0.76
I ₂	21	5.39	0.21	7.91	0.45	16	6.06	0.24	9.64	0.65
C ₁	44	9.40	0.43	6.42	0.33	28	12.04	0.91	9.54	0.87
P ₃	32	10.62	0.41	6.74	0.23	27	13.13	0.82	8.23	0.19
P ₄	35/34	7.89	0.65	9.08	0.52	32/33	9.49	0.62	10.48	0.37
M ₁	35	10.57	0.48	9.63	0.37	33	12.20	0.53	11.18	0.48
M ₂	40	12.12	0.56	11.30	0.36	49	14.51	0.59	13.23	0.60
M ₃	44/43	12.69	0.67	11.14	0.54	25	15.03	0.77	12.90	0.64

differences) more similar to those between the sexes in extant apes (average of 20 statistically significant differences)^{25,26}, and in *Ramapithecus* (with 9 statistically significant differences) somewhat closer to that of the smaller sexual dimorphism of humans (5 significant differences)²⁷. Likewise, the degree of discreteness between the two peaks (when they exist) in each genus is generally much greater in *Sivapithecus* than in *Ramapithecus* and again generally models the differences between the sexes in apes and humans, respectively. The clarity of the separations between the peaks where they exist is also generally considerably greater in breadth measurements in each group than in the corresponding measurements of length (a finding that is, in general true for many breadth measurements between the sexes of the teeth of many primates²⁵, and one that has also recently been shown to apply to breadth measurements from whatever part of the body they may be taken²⁸). Finally, the two peaks in the frequency distribution histograms, when present, are generally of equal size in *Ramapithecus* but are usually unequal in *Sivapithecus*; in this latter group the peak representing the larger specimens comprises a much smaller proportion of them (Fig. 3). If these differences are truly due to sexual dimorphism, then *Ramapithecus* may be displaying equal numbers of teeth from males and females, and *Sivapithecus* a male/female ratio that ranges between approximately 2:1 to 4:1. This may well mean that *Ramapithecus* possesses a similar nuclear family social sexual structure to that which exists in humans, and *Sivapithecus* a polygynous one like all extant great apes.

Individual scientists visiting China and examining the fossils visually doubt the separation of the cranial specimens into *Ramapithecus* and *Sivapithecus*. For instance, it has been suggested to one of us (W. R.) that the two groups found at Lufeng are merely the males and females of an ape-like species, possibly an orang-utan ancestor, with very marked sexual dimorphism. This idea seems untenable on the basis of the new evidence.

Because, in these patterns, *Ramapithecus* somewhat resembles modern humans and *Sivapithecus* resembles all of the modern great apes, it is tempting to draw the obvious implication for the lineages of these forms. Such implications could certainly be made immediately, for instance, if we relied upon the conventional view that structural sexual dimorphism is basically a single phenomenon within the primates, mainly associated with size^{29,30} and that it is displayed to greater degrees in polygynous social organizations and to lesser degrees in nuclear family social organizations^{31,32}.

However, the recent finding that structural sexual dimorphism may include as many as five different patterns among the living

hominoids alone and many more when all primates are included, and that it is clearly related to phenomena other than simply size (and size-related shape) alone²⁸, requires that we be far more circumspect in interpreting these data. The two patterns described above may not be the only differences between the sexes in these two forms. For instance, the data at hand suggest that there are patterns along the tooth row that differ both between *Ramapithecus* and *Sivapithecus*, and from each of the living hominoids^{33,34}. This work may eventually allow us to determine whether any of the known forms of sexual dimorphism in extant hominoids also apply to either of these fossils, or whether yet other unique sexual dimorphisms exist within them. Our continued investigation of these teeth may provide further evidence about the phylogenetic relationships sampled here. It is certain, however, that the present data are far firmer than those so far known for all other ramapithecine fossil finds combined.

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- Lewis, G. E. *Am. J. Sci.* **22**, 161–181 (1934).
- Leakey, L. A. *Mag. nat. Hist.* **13**, 689–696 (1962).
- Simons, E. *Early Hominids of Africa* 543–566 (Duckworth, London, 1978).
- Kretzoi, M. *Nature* **257**, 578–581 (1975).
- Von Koenigswald, G. H. R. *Proc. K. ned. Akad. Wet.* **B75**, 385–394 (1972).
- Andrews, P. & Tobien, H. *Nature* **268**, 699–701 (1977).
- Simons, E. & Pilbeam, D. *Folia Primatol.* **3**, 81–152 (1965).
- Simons, E. *Scient. Am.* **236**, 28–35 (1977).
- Smith, R. J. & Pilbeam, D. *Nature* **284**, 447–448 (1980).
- Lipson, S. & Pilbeam, D. *J. hum. Evol.* **11**, 545–548 (1982).
- Andrews, P. & Cronin, J. E. *Nature* **297**, 541–546 (1982).
- Todd, M. P. *Folia Primatol.* **38**, 141–157 (1982).
- Ward, S. C. & Kimbel, W. H. *Am. J. phys. Anthropol.* **61**, 157–172 (1983).
- Wu, R. *Vert. Palas.* **1**, 25–32 (1957).
- Wu, R. *Vert. Palas.* **2**, 38–43 (1958).
- Wu, R. *Palaeont. Sinica* **11**, 65–94 (1962).
- Xu, Q. *China Reconstructs* **29**, 45–57 (1981).
- Wu, R. *Recent Advances of Chinese Palaeontology* (University of Hong Kong Press, 1982).
- Yip, C. *Fossil Man in China* (Hong Kong Museum of History, Hong Kong, 1983).
- Xu, Q. & Lu, Q. *Vert. Palas.* **17**, 173 (1979).
- Xu, Q. & Lu, Q. *China Reconstructs* **29**, 56–57 (1980).
- Wu, R. *et al. Kexue Tongbao* **26**, 1018–1021 (1981).
- Wu, R. *et al. Act. anthropol. et al. sinica* **1**, 101–108 (1982).
- Wu, R., Xu, Q. & Lu, Q. *Acta anthropol. Sinica* **2**, 1–14 (1983).
- Swindler, D. R. *Dentition of Living Primates* (Academic, London, 1976).
- Ashton, E. H. & Zuckerman, S. *Proc. R. Soc. B234*, 471–483 (1950).
- Lavelle, C. L. B. *J. dental Res.* **47**, 811–815 (1968).
- Oxnard, C. E. *Am. J. Primatol.* **4**, 169–191 (1983).
- Wood, B. A. thesis, Univ. London (1975).
- Wood, B. A. *J. Zool. Lond.* **180**, 15–34 (1976).
- Fliegler, J. G., Kay, R. F. & Simons, E. *Nature* **287**, 328–330 (1980).
- Gingerich, P. D. *Am. J. phys. Anthropol.* **56**, 217–234 (1981).
- Wu, R. & Oxnard, C. E. *Am. J. Primatol.* **5**, 303–343 (1983).
- Oxnard, C. E. *The Order of Man* (Yale University, New Haven and Hong Kong University, Hong Kong, 1983).

Sexual selection by male-male competition in natterjack toad choruses

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A major controversy of sexual selection theory is whether sexually dimorphic characters used in display by males have arisen through male-male competition, female choice, or both¹⁻⁴. Here I describe the chorusing behaviour of male natterjack toads (*Bufo calamita*) and show that the pitch of the advertisement call is an important determinant of male mating success. Playback experiments using synthetic calls show that when males compete for calling sites they use call pitch to assess the body size and fighting ability of their opponents. Although females initiate mating and can potentially choose any calling male, in two-choice playback experiments, they showed no mating preference based on call pitch. Several authors⁴⁻⁶ have suggested that communal sexual displays by males may have a more important role in intrasexual competition than mate choice, but this is the first time that this hypothesis has been tested experimentally by manipulating a signal property important in male display.

In Britain, natterjack toads breed from late March to June in shallow ponds between sand dunes. The study sites were ponds in the Ainsdale Sand Dunes National Nature Reserve, Merseyside and Eskmeals Nature Reserve, Cumbria where I made observations during 1980-83. Adult males formed choruses and called loudly from the edge of the pond for 4-6 h after dusk, and on still nights the chorus could be heard 2 km away. The advertisement call is a simple trill with a fundamental frequency (pitch) of 1,200-1,830 Hz (median = 1,500 Hz, $n = 37$), repeated once every second at 12 °C. As in other anurans⁷⁻¹² call pitch is inversely correlated with male body length ($r = -0.571$, $n = 21$, $P < 0.01$), but does not vary with temperature. Females respond to the call phonotactically, and were sometimes found moving towards a chorus from over 1 km away. After arriving at the pond, females ($n = 52$) spent from 30 s to 4 h in the water, swimming close to several calling males and adopting a characteristic 'listening' posture with the body axis vertical in the water and the tympanum clearly visible above the surface. Eventually, they swam up to a calling male and nudged him to elicit amplexus. Spawning usually took place some distance (2-50 m) from the male's calling site within 24 h of pairing. Males, therefore, do not offer any resources to the female and the mating system is similar to leks^{13,14} in birds and mammals.

The mating success of a small population ($n = 21$) of males was monitored at a pond in the Ainsdale reserve in spring 1980. I identified male toads individually by natural variations in the pattern of the yellow vertebral stripe and the position of 'warts' on the skin. Every night during the breeding season (8 weeks) I noted the presence or absence of each male at the pond and recorded body length, weight, the pitch and amplitude of the calls, calling activity, distance from other calling males and weight loss.

The most successful male mated with seven different females during the season whereas two males failed to mate at all. The mean mating success of males was 2.48 mates, reflecting a female-biased sex ratio which was probably a consequence of greater male mortality during the breeding season (due to bird predation). The number of nights each male visited the pond was the single most important factor affecting mating success, accounting for 51% of the total variance in male success ($r = 0.714$, $n = 15$, $P < 0.01$). Male mating success was also inversely correlated with call pitch ($r = -0.588$, $n = 15$, $P < 0.05$), sug-

Table 1 Factors affecting mating success of calling males (dependent variable, mating success)

Independent variables	Simple r ($n = 15$)	Standardized partial regression coefficient, β
No. of nights at pond	0.714†	1.16‡
Body length	0.328	-0.21
Body weight	0.454	0.25
Call pitch	-0.588*	-0.60*
Call amplitude	0.386	-0.02
Mean distance to nearest caller	0.241	0.33
% Time calling	0.505	0.20
% Weight loss	0.441	-1.07
Multiple $R^2 = 0.91$	s.s.	d.f.
Regression	53.32	8
Residual	4.41	6
	m.s.	F
	6.67	9.06†
	0.74	—

Six non-calling males who obtained females by intercepting them as they were approaching callers are excluded from the analysis. s.s., Sum of squares; m.s., mean square.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

gesting that males with low-pitch calls enjoyed greater success than those with high-pitch calls.

Although simple correlation analysis is often used to investigate causes of variation in mating success, several problems arise when traits are intercorrelated^{15,16}. Because there is a strong correlation between call pitch and male size it is impossible to know whether selection acts directly on call pitch, or whether the relationship between call pitch and mating success is merely a correlated response to selection on male size. I eliminated the complication of correlations between independent variables by calculating the partial regression of relative mating success on each measured variable, holding all other variables constant. The results of this analysis (Table 1) show that call pitch has a direct effect on mating success whereas the effect of male size is not significant.

Call pitch may affect mating success through male-male competition¹¹, female choice¹², or both. Although male natterjacks rarely fight for already-paired females they attempt to drive rival callers away from the vicinity of their own calling sites and maintain an exclusive zone of attraction for females. Males frequently stopped calling, swam up to a rival calling male and 'clasped' him firmly in an axillary embrace. Calling males were most likely to be displaced from calling sites by attackers larger than themselves (45 out of 56 encounters), but more likely to resist displacement when the attacking male was smaller (26 out of 36 encounters; $\chi^2 = 23.1$ corrected for continuity, d.f. = 1, $P < 0.001$). There was a significant relationship between a male's body length and his distance from other callers averaged over the breeding season ($r = 0.592$, $n = 21$, $P < 0.01$), reflecting the greater success of large males in fights for calling sites. In 56 out of 92 fights the attacking male was larger than the calling male, indicating that males were significantly more likely to initiate attacks on calling males smaller than themselves than would be expected by chance ($P < 0.05$, two-tailed binomial test).

I tested the hypothesis that males use call pitch to assess the size of their calling rivals prior to an attack by playing synthetic (that is, electronically-generated)¹⁷ advertisement calls to males though a small loudspeaker. I used synthetic rather than natural calls in order to vary pitch while holding other parameters constant. One low-pitch call (1,200 Hz) and one high-pitch call (1,800 Hz) were used, representing the largest and smallest males found at the study site, and the calls were broadcast for 5 min at uniform intensity ~1 m from each calling male. The behavioural responses to the playbacks were variable but there was a significant difference between the distribution of responses in each treatment (Table 2; $\chi^2 = 18.5$, d.f. = 2, $P < 0.001$). The proportion of males swimming away from the loudspeaker was

Table 2 Responses of calling males to playbacks of synthetic calls

	No. of males tested	Response			
		Swam away	Continued calling	Stopped calling	Attacked loudspeaker
1,800 Hz	36	3	7	4	22
1,200 Hz	35	18	4	6	7

Table 3 Number of choices made by females in simultaneous two-choice tests using synthetic calls

	No. of females tested	Choices		P (two-tailed binomial test)
a	17	1,300 Hz	1,600 Hz	0.63 (1.00)
		10 (14)	7 (13)	
b	8	1,200 Hz	1,800 Hz	0.29 (0.27)
		2 (4)	6 (9)	
c	8	80 dB	82 dB	0.008 (0.001)
		0 (0)	8 (12)	
d	12	81 dB (perceived 75 dB)	75 dB (perceived 75 dB)	1.00 (0.80)
		6 (7)	6 (9)	

a–b, Call pitch varied; c–d, call amplitude varied.

Values given in columns 2 and 3 without parentheses are numbers of females choosing each call in their first trial; some females were given more than one trial and values in parentheses are total numbers of choices made combining all trials.

significantly greater during playback of the low-pitch call ($\chi^2 = 13.8$ corrected for continuity, d.f. = 1, $P < 0.001$) whereas the proportion of males attacking (jumping on the loudspeaker) was greater for the high-pitch call ($\chi^2 = 10.7$ corrected for continuity, d.f. = 1, $P < 0.01$). The results are consistent with the notion that call pitch is a signal of body size used by males to assess the fighting ability of other callers¹¹.

To test whether females use call pitch in mate choice I presented gravid females with a choice of two synthetic calls differing only in pitch and played them back simultaneously at equal amplitude. Females readily approached the loudspeakers, but failed to show any significant preference (Table 3). Moreover, 9 out of 14 females tested a second time with the same calls chose the opposite call to their first trial choice. Thus, choices based on call pitch were inconsistent both between females and between trials on single females.

Could females use any other cue to choose a mate of a certain size? Call amplitude is the only other vocal cue correlated with male size ($r = 0.806$, $n = 32$, $P < 0.001$) but it is unlikely to be a reliable predictor of male size because vegetational and microclimatic variations in different parts of the pond will affect call attenuation¹². Furthermore, females must assess distances to calling males to compute the absolute call amplitude (and therefore the size) of each potential mate. In further playback experiments females were given a choice between two calls differing in amplitude by 2 dB and played back at equal distance from the female. All of eight females chose the louder call in their first trial. However, when the loudspeakers were placed at different distances from the female so that call amplitudes perceived by the female were equal but the absolute amplitudes (measured at 1 m from each loudspeaker) differed by 6 dB, females did not show a significant preference (Table 3). These results imply that females are simply passively attracted¹⁸ to the call perceived at the highest amplitude and do not actively choose the call with the highest absolute amplitude.

These playback experiments suggest that call pitch influences a male's mating success via male–male competition rather than from female choice. It is unlikely that female natterjacks would benefit by mating with a large male rather than a small one. Unlike some other species^{12,19}, male size does not influence fertilization success (A.A., in preparation), nor developmental success of offspring²⁰, and it seems doubtful that females could obtain indirect benefits by passing on genes for rapid growth or high survival to their offspring³, assuming these traits are heritable²¹. Although heritability of body size is not known for any anuran, it is probably low in *B. calamita*, as in other species where conditions for growth and survival are highly variable²².

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1. Darwin, C. *The Descent of Man, and Selection in Relation to Sex* (Murray, London, 1871).
2. Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon, Oxford, 1930).
3. Trivers, R. L. in *Sexual Selection and the Descent of Man* (ed. Campbell, B.) 136–179 (Heinemann, London, 1972).
4. Halliday, T. R. in *Mate Choice* (ed. Bateson, P.) 3–32 (Cambridge University Press, 1983).
5. LeCroy, M., Kulupi, A. & Peckover, W. S. *Wilson Bull.* **92**, 289–301 (1980).
6. Diamond, J. M. *Nature* **293**, 257–258 (1981).
7. Blair, W. F. Q. *Rev. Biol.* **39**, 334–344 (1964).
8. Porter, K. R. *Am. Midl. Nat.* **71**, 232–245 (1964).
9. Zweifel, R. G. *Copeia* **1968**, 269–284 (1968).
10. Nevo, E. & Schneider, H. *J. Zool.* **178**, 133–145 (1976).
11. Davies, N. B. & Halliday, T. R. *Nature* **274**, 683–685 (1978).
12. Ryan, M. J. *Evolution* **37**, 261–272 (1983).
13. Halliday, T. R. in *Behavioural Ecology: An Evolutionary Approach* (eds Krebs, J. R. & Davies, N. B.) 180–213 (Blackwell, Oxford, 1978).
14. Bradbury, J. W. & Gibson, R. M. in *Mate Choice* (ed. Bateson, P.) 109–138 (Cambridge University Press, 1983).
15. Lande, R. *Evolution* **33**, 402–416 (1979).
16. Arnold, S. J. in *Mate Choice* (ed. Bateson, P.) 67–107 (Cambridge University Press, 1983).
17. Gerhardt, H. C. *J. exp. Biol.* **61**, 229–241 (1974).
18. Parker, G. A. in *Current Problems in Sociobiology* (ed. King's College Sociobiology Group) 173–201 (Cambridge University Press, 1982).
19. Davies, N. B. & Halliday, T. R. *Nature* **269**, 56–58 (1977).
20. Howard, R. D. *Ecology* **59**, 789–798 (1978).
21. Maynard-Smith, J. *The Evolution of Sex* (Cambridge University Press, 1978).
22. Borgia, G. *Anim. Behav.* **29**, 71–80 (1981).

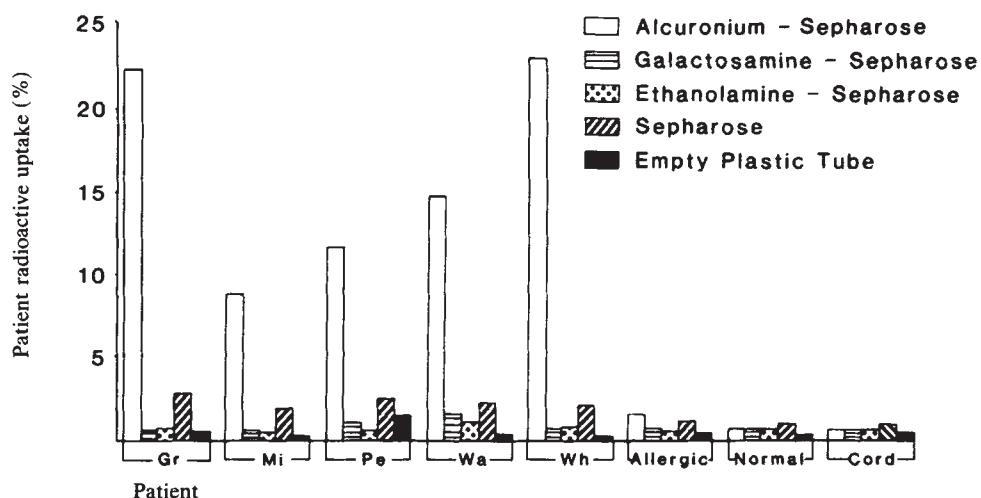
Substituted ammonium ions as allergenic determinants in drug allergy

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Serious, and occasionally fatal, anaphylactic-like (anaphylactoid) reactions may occur when a patient is exposed to a drug for the first time¹. Apart from the penicillins², nothing is known of the nature of antigenic or sensitizing drug determinants and, as yet, there is no evidence for the involvement of IgE antibodies in most drug reactions^{1,3}. Muscle relaxants such as alcuronium have been implicated in many life-threatening anaphylactoid reactions^{4,5} but the mechanisms remain unclear⁶. We have now investigated the possibility that drug-specific IgE antibodies are involved by using an alcuronium-carrier complex in a radioimmunoassay with patients' sera. Alcuronium-reactive antibodies were found in five drug-sensitive subjects and most of the antibodies cross-reacted with other muscle relaxants and with a variety of apparently structurally unrelated drugs. Structure-activity studies designed to explore the molecular basis of the antibody binding established that quaternary and tertiary ammonium ions were the complementary allergenic sites on the reactive drugs. These structures occur widely in many drugs but also in foods, cosmetics, disinfectants and industrial materials. Hence, there would seem to be ample opportunity for sensitive individuals to come into contact with and synthesize IgE antibodies to these unusual, and previously unsuspected, antigenic determinants.

Fig. 1 Representation of reaction of human IgE antibodies in patients' sera with alcuronium-Sephadex complex and with control solid phases. The presence of specific IgE antibodies was determined by the per cent radioactive uptake of ^{125}I -anti-IgE. For the five different solid supports, alcuronium-Sephadex, D-galactosamine-HCl-Sephadex, ethanolamine-Sephadex, Sephadex alone and empty plastic tubes, the mean uptakes with the 12 allergic sera were 1.6 ± 0.4 , 0.7 ± 0.2 , 0.5 ± 0.1 , 1.3 ± 0.3 and 0.3 ± 0.05 , respectively. Mean uptakes with the 20 normal sera were 1.1 ± 0.3 , 1.0 ± 0.3 , 1.0 ± 0.2 , 1.2 ± 0.4 and 0.2 ± 0.04 , respectively. Patient's serum (50 μl) or serum from cord blood (IgE-free control), a normal (non-allergic) subject or an allergic patient with high levels of IgE to some common allergens, was added to the solid phase (6 mg) and incubated at room temperature for 3 h before washing three times with phosphate-buffered saline containing 0.5% Tween 20 (PBS-Tween). Immunoabsorbent-purified anti-human IgE labelled with ^{125}I to a specific activity of $\sim 1.25 \mu\text{Ci}$ per μg (Pharmacia) ($30,000$ – $50,000$ c.p.m. per tube) was added and tubes were left overnight at room temperature before washing four times with PBS-Tween and counting. Solid-phase complexes were prepared by reacting alcuronium (Roche) (60 mg), D-galactosamine-HCl (Sigma) (60 mg) or ethanolamine (BDH) (600 mg) with epoxy-activated Sephadex (Pharmacia)¹⁴. Compounds were dissolved in 5 ml of distilled water and adjusted to pH 12 with 2.5 M NaOH before adding to washed activated Sephadex. Following gentle shaking at 30°C for 20 h and successive washings with water, 0.1 M borate buffer pH 8 and 0.1 M acetate buffer pH 4, remaining free activated groups were blocked at room temperature for 4 h by the addition of 10 ml of 1 M ethanolamine pH 9. The conjugated Sephadex beads were washed as above and then with distilled water before use in the radioimmunoassay.



A recent study⁷ showed that 4% of hospital admissions were due to adverse reactions to therapeutic doses of drugs and almost half of these were attributed to anaphylactoid reactions. Patients' reactions are frequently described as 'idiosyncrasies' although this term tells us nothing about underlying mechanisms. Anaphylactoid reactions are so described because they mimic allergic reactions induced by IgE and allergen⁸, although there is little evidence of true type 1, IgE-mediated drug reactions and nothing is known of the nature of the antigenic determinants on sensitizing drugs. This ignorance provides a virtually insurmountable barrier to immunological study of adverse drug reactions⁹.

Following the finding of positive intradermal and passive transfer tests to muscle relaxants^{10,11}, the difficulty of accounting for the specificity of the drug reactions by an idiosyncratic mechanism, and recognition that insufficient histamine is released by these drugs in nominal dosage to make severe reactions likely^{6,12}, we investigated the possibility that sensitive patients experienced a type 1, IgE-mediated drug reaction. Sera taken from five patients, Gr, Mi, Pe, Wa and Wh, after they had experienced an anaphylactoid reaction following intravenous administration of muscle relaxants, were examined for drug-specific IgE antibodies together with cord serum and sera from 20 'normal' (non-allergic) and 12 allergic subjects. The drug-solid support complex used in the assay was prepared by coupling the muscle relaxant alcuronium directly to Sephadex via a 'spacer arm' (Fig. 1), thus avoiding the need first to make the drug antigenic by linking to a carrier protein. Sera from alcuronium-sensitive patients produced high uptakes of radioactive anti-IgE (9–23%) but uptakes recorded with all other sera were only 0.2–1.5% (Fig. 1). Specificity of the assay was further checked using D-galactosamine HCl- and ethanolamine-Sephadex complexes, Sephadex and empty plastic tubes¹³. With each of these four different test systems, uptakes of ^{125}I -anti-IgE were generally less than 1% and never more than 2.8% (Fig. 1). From the direct binding radioimmunoassay studies we concluded that sera from the five drug-sensitive subjects contained alcuronium-reactive IgE antibodies.

This conclusion was confirmed by experiments in which patients' sera were preincubated with free alcuronium before mixing, first with alcuronium-Sephadex complex and then, after washing, with labelled anti-IgE. Inhibition experiments of this type revealed that alcuronium inhibited binding of all of the

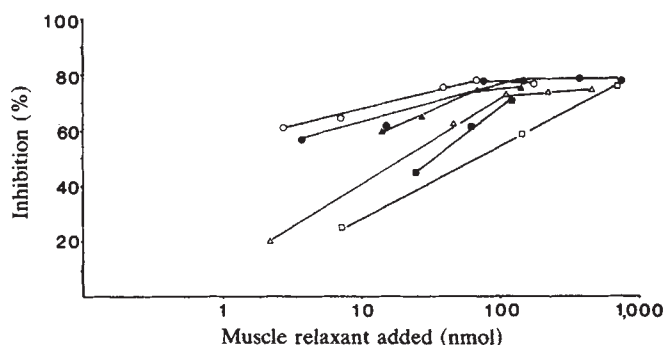


Fig. 2 Inhibition by muscle relaxant drugs of the binding of IgE antibodies to alcuronium-Sephadex complex. Serum Gr diluted 1:8 (50 μl) was incubated with drug in a total volume of 100 μl for 1 h before the addition of 6 mg of alcuronium-Sephadex in 100 μl of water. Three hours later, each tube was centrifuged at 3,500 r.p.m. for 10 min, the supernatant removed by aspiration and the sediment washed three times by addition of PBS-Tween. ^{125}I -Anti-human IgE (50 μl) was added and the tubes were left overnight before washing four times with PBS-Tween. Percentage inhibition was calculated with reference to the radioactive uptakes in tubes where serum Gr was preincubated with saline instead of drug. ○, Alcuronium; ●, (+)tubocurarine; □, succinylcholine; ■, decamethonium; △, gallamine; ▲, pancuronium.

sera, but two samples, Gr (Fig. 2) and Wh, cross-reacted strongly with each of five other muscle relaxants—decamethonium, gallamine, pancuronium, suxamethonium and (+)tubocurarine. Sera Mi and Pe also reacted with the same five drugs but less strongly than Gr and Wh. By contrast, serum Wa reacted only with alcuronium. In an attempt to understand the basis for the cross-reactions observed with sera Gr, Mi, Pe and Wh, the combining sites of these sera were studied in greater detail. As conclusions from experimental findings with each were similar, detailed results are presented for only one serum (Gr).

Apart from the presence of quaternary ammonium groups in each of the six muscle relaxants tested, the compounds show little structural similarity. Hence, the most likely chemical basis for the observed cross-reactivities seemed to be the quaternary ammonium ion. This was further investigated by testing several quaternary ammonium compounds including choline and acetylcholine halides, some tetraalkylammonium bromides and

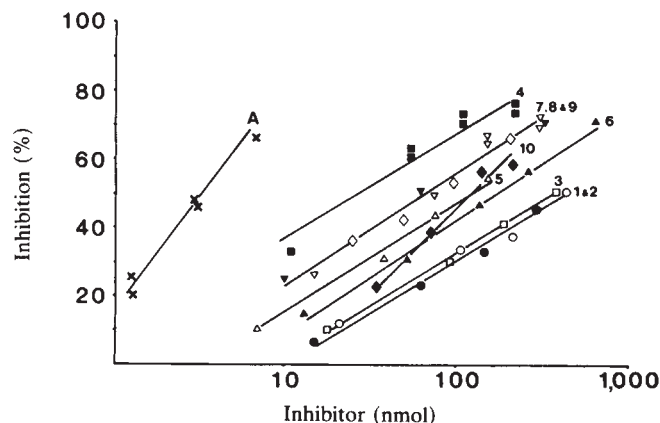


Fig. 3 Inhibition by quaternary ammonium compounds of the binding of IgE antibodies in serum Gr to alcuronium-Sephadex complex. For details of assay see Fig. 2 legend. 1, O, Choline chloride; 2, ●, acetylcholine bromide; 3, □, tetramethylammonium bromide; 4, ■, tetrapropylammonium bromide; 5, △, tetrapentylammonium bromide; 6, ▲, ethyltrimethylammonium iodide; 7, ▽, octyltrimethylammonium bromide; 8, ▼, decyltrimethylammonium bromide; 9, ◇, dodecyltrimethylammonium bromide; 10, ◆, hexadecyltrimethylammonium bromide; A, alcuronium.

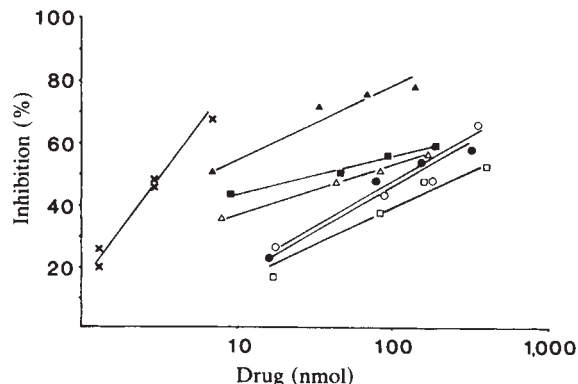


Fig. 4 Inhibition of some compounds containing tertiary or quaternary ammonium ions, and with different pharmacological activities, of the binding of IgE antibodies in serum Gr to alcuronium-Sephadex. For details of assay see Fig. 2 legend. O, Promethazine-HCl; ●, chlorpromazine-HCl; □, neostigmine bromide; ■, pentolineum tartrate; △, trimethaphan camphorsulphonate; ▲, morphine-HCl; ×, alcuronium.

a series of alkyltrimethylammonium salts. Each of the compounds inhibited binding of serum Gr IgE to alcuronium-Sephadex (Fig. 3). IC_{50} ratios relative to alcuronium ranged from 8 for tetrapropylammonium bromide to 130 for choline and acetylcholine. Inhibition studies were extended to include a large number of other N-containing compounds, including primary, secondary and tertiary amines. No inhibition with up to 1,000 nmoles per tube was observed with, for example, tris(hydroxymethyl)aminomethane-HCl, arginine, lysine, taurine, ethanolamine, diethanolamine, triethanolamine and galactosamine-HCl, but promethazine-HCl, an ammonium salt of a phenothiazine derivative with no muscle relaxant properties, produced 50% inhibition at a concentration of 120 nmoles (Fig. 4). We therefore examined other pharmacologically active compounds without muscle relaxant activity but containing either a quaternary or a tertiary ammonium ion, and found that IgE antibodies in serum Gr also reacted with such structurally diverse compounds as neostigmine bromide, pentolineum tartrate, trimethaphan camphorsulphonate, chlorpromazine-HCl and morphine-HCl (Fig. 4). The latter compound was particularly active, requiring only 6–7 nmoles for 50% inhibition.

Since completion of the analysis of the above 5 sera, 13 additional cross-reacting positive sera from patients who experienced life-threatening anaphylactoid reactions to muscle relaxants have been found. We conclude, therefore, that IgE antibodies to quaternary ammonium muscle relaxant drugs occur in the sera of at least some patients who experienced life-threatening anaphylactic-like reactions during general anaesthesia. Most of these antibodies show marked cross-reactivity with a variety of other compounds that have quaternary or tertiary ammonium ions in the molecule. Included in the list of reactive or potentially reactive structures are some frequently used, pharmacologically active drugs such as antihistamines, anti-anxiety agents, antihypertensives, parasympathomimetics and narcotics. The long list of drugs containing a substituted ammonium ion makes it likely that many more frequently prescribed drugs will be found to react with IgE in the sera of patients with drug allergies.

Although implications of our findings and underlying mechanisms (for example, allergic sensitization and triggering) have yet to be assessed and understood, we have shown that previously unsuspected structures forming only part of small molecules that are neither peptide, carbohydrate nor lipid in nature may be complementary to IgE, the antibody involved in human allergic reactions. Our results also provide insights into the size

of some IgE combining sites, a subject that has so far been little studied.

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1. Patterson, R. & Anderson, J. *J. Am. med. Ass.* **248**, 2637–2645 (1982).
2. Ahlstedt, S. & Kristofferson, A. *Prog. Allergy* **30**, 67–134 (1982).
3. Ackroyd, J. F. in *Clinical Aspects of Immunology* 3rd edn (eds Gell, P. G. H., Coombs, R. A. & Lachmann, P. J.) 913–961 (Blackwell, Oxford, 1975).
4. Lim, M. & Churchill-Davidson, H. C. *Monogr. Anaesth.* **8**, 65–136 (1981).
5. Fisher, M. M. & More, D. *Anaesth. intensive Care* **9**, 226–234 (1981).
6. Fisher, M. M. & Munro, I. *Anaesth. Analg.* **62**, 559–564 (1983).
7. Levy, M., Lipshitz, M. & Eliakim, M. *Am. J. med. Sci.* **177**, 49–56 (1979).
8. Beaven, M. A. *Anesthesiology* **55**, 3–5 (1981).
9. Adkinson, N. F. & Lichtenstein, L. M. *Alfred Benzon Symp.* **10**, 123–140 (1977).
10. Fisher, M. M. *Anesthesiology* **52**, 318–320 (1980).
11. Fisher, M. M. *Anaesth. intensive Care* **9**, 235–241 (1981).
12. Mongar, J. F. & Whelan, R. F. *J. Physiol., Lond.* **120**, 146–150 (1953).
13. Baldo, B. A., Wells, I. D. & Pepys, J. *Clin. Allergy* **4**, 195–210 (1974).
14. Sundberg, L. & Porath, J. *J. Chromatogr.* **90**, 87–98 (1974).

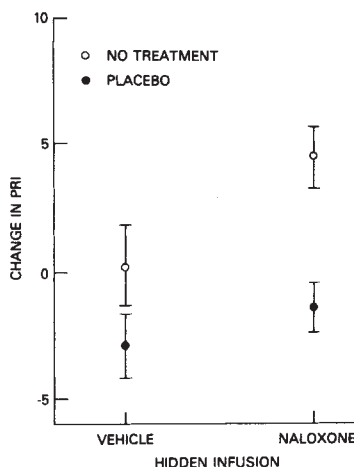
Placebo and naloxone can alter post-surgical pain by separate mechanisms

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The discovery of an endogenous opioid-mediated analgesic system^{1,2} has led to the search for its physiological roles and how it might be activated in natural conditions. Environmental and surgical stress and certain forms of transcutaneous electrical stimulation or acupuncture appear to activate this system^{3–5}. Several studies also suggest that this opioid system mediates placebo analgesia. Placebo reduces post-surgical pain in comparison with no treatment⁶, and this analgesia is apparently reversed by the opioid antagonist, naloxone^{7–8}. However, these studies did not indicate whether naloxone and placebo exert their effects by common or by separate mechanisms. By administering hidden infusions of naloxone (in subjects unaware that the medication was being given) separate from the administration of a placebo, we were able to assess the effects of these two treatments independently. We report here evidence that placebo analgesia can occur after blockade of opioid mechanisms by naloxone and that naloxone can produce hyperalgesia independent of the placebo effect. The combined action of these effects is sufficient to explain the reversal of placebo analgesia by naloxone.

Fig. 1 Effect of placebo and naloxone after 60 min. Change in the pain rating index (PRI) of the McGill pain questionnaire from the 10-min pretreatment baseline is shown for the groups receiving 'hidden' infusions of naloxone vehicle (left) and groups receiving 'hidden' infusions of naloxone (right). Groups receiving no subsequent 'known' treatment are shown by the open circles, groups receiving a subsequent 'known' infusion of saline placebo are shown by the filled circles. Baseline scores were: naloxone vehicle, no treatment, 20.9; naloxone vehicle, placebo, 16.1; naloxone, no treatment, 18.1; naloxone, placebo, 17.4. Both naloxone and placebo produced significant and independent effects.



Hidden infusions of naloxone (10 mg) or naloxone vehicle were administered double-blind through an indwelling intravenous line immediately before the double-blind administration of 0.11 $\mu\text{g kg}^{-1}$ fentanyl, saline placebo, or no treatment. The fentanyl groups were included to assess the sensitivity of the measurements to the reversal of opiate analgesia by naloxone. Eighty-nine dental patients received these treatments 2 h after extraction of an impacted lower third molar tooth under 2% lidocaine anaesthesia without a vasoconstrictor. Unlike previous studies, nitrous oxide and diazepam sedation were not used to avoid possible interaction between naloxone and these agents. This procedure results in significant post-operative pain 2 h after surgery that is comparable with that found 4 h after surgery in sedated patients⁶.

Patients completed the McGill pain questionnaire 60 and 10 min before and after the experimental treatments⁹. The 60-min pretreatment assessment served to familiarize the patients with the questionnaire. The effects were calculated by subtracting the post-treatment pain rating index (PRI) scores from the 10-min pre-treatment baseline.

Analysis of the fentanyl groups showed that the PRI was sensitive to the effects of fentanyl and naloxone. Baseline PRI scores in the fentanyl and fentanyl-naloxone groups were 17.44 and 16.88, respectively. The reductions in pain produced by fentanyl alone at 10 min (-10.83) and 60 min (-4.61) were reversed when naloxone was given with fentanyl (10 min, -0.40; 60 min, +2.8). These differences were significant at both 10 min ($t(31) = 4.25$, $P < 0.001$) and 60 min ($t(31) = 2.19$, $P < 0.05$).

The effects of hidden naloxone and placebo were assessed by an analysis of covariance to reduce the influence of baseline levels. A two-way analysis of the hidden infusion (naloxone or naloxone vehicle) versus the known treatment (no treatment or placebo) assessed the separate effects of naloxone and placebo and the interaction of these effects. Figure 1 shows the effects of naloxone and placebo at 60 min. Pain was significantly decreased after placebo ($F(1,51) = 12.45$, $P < 0.001$), as shown by the difference between the open and filled circles. Pain was significantly increased after the administration of naloxone ($F(1,51) = 5.27$, $P < 0.05$), as shown by the differences between the left and right points. There was no significant interaction between these effects ($F(1,51) = 1.16$, $P = 0.29$), indicating that the effects of placebo and naloxone were independent.

The left points in Fig. 1 show a typical placebo effect. Placebo reduced pain in comparison with no treatment ($t(27) = 2.04$, $P < 0.05$). The right points demonstrate that this effect also occurred after administration of naloxone ($t(25) = 3.75$, $P < 0.001$). These findings show that placebo effects can occur independently of opioid mechanisms. Other analgesia-producing environmental manipulations, such as hypnosis^{10,11}

and some forms of transcutaneous electrical nerve stimulation^{12,13}, also are not opioid-mediated. Figure 1 also shows a trend for naloxone to increase post-surgical pain after administration of placebo, as shown by the difference between the filled circles. This is the effect found in previous studies⁶⁻⁸. However, the difference between the open circles shows that naloxone also increased post-surgical pain ($t(22) = 2.32$, $P < 0.05$) in the no-treatment condition. Thus, naloxone hyperalgesia does not depend on placebo administration. As this hyperalgesia is similar in the no-treatment and placebo groups, there is no evidence for an opioid component to placebo analgesia in this study. This finding suggests that naloxone hyperalgesia may involve the antagonism of endogenous opioid compounds released as a consequence of surgical stress. Although naloxone reverses stress analgesia^{3,14}, it has no effect in pain-free subjects¹⁵, supporting the interpretation that opioid activity was minimal before surgery and was increased by surgical stress. However, we emphasize that naloxone hyperalgesia, especially in the high doses used in this and other studies, may result from the antagonism of other putative neurochemical mediators¹⁶ or some other mechanism.

We conclude that at least two different mechanisms, one opioid related and one non-opioid related, may be influencing post-surgical pain in the present study. We have shown that significant placebo analgesia can occur after blockade of opioid mechanisms by naloxone and that naloxone can produce hyperalgesia independent of the placebo effect. The combined action of these effects is sufficient to explain the increase in pain in comparison with placebo produced by naloxone as well as its apparent reversal of placebo analgesia⁶⁻⁸. The absence of an opioid component to placebo analgesia in this study does not rule out its presence in other situations in which placebos are administered. However, our experimental design clarifies the conditions necessary to demonstrate such an effect.

Received 7 July; accepted 30 August 1983.

1. Snyder, S. H. & Childers, S. R. *Rev. Neurosci.* **2**, 35-64 (1979).
2. Yaksh, T. L. & Rudy, T. A. *Pain* **4**, 299-359 (1978).
3. Watkins, L. R. & Mayer, D. J. *Science* **216**, 1185-1192 (1982).
4. Cohen, M. *et al. Lancet* **1**, 213-214 (1981).
5. Mayer, D. J., Price, D. D. & Rafii, A. *Brain Res.* **121**, 368-372 (1977).
6. Gracely, R. H. *et al. Soc. Neurosci. Abstr.* **5**, 609 (1979).
7. Levine, J. D., Gordon, N. C. & Fields, H. L. *Lancet* **ii**, 654-657 (1978).
8. Levine, J. D., Gordon, N. C., Jones, R. T. & Fields, H. L. *Nature* **272**, 826-827 (1978).
9. Melzack, R. *Pain* **1**, 277-299 (1975).
10. Barber, J. & Mayer, D. *Pain* **4**, 41-48 (1977).
11. Goldstein, A. & Hilgard, E. R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2041-2043 (1975).
12. Chapman, C. R., Colpitts, Y. M., Benedetti, C., Kitaef, R. & Gehrig, J. D. *Pain* **9**, 183-197 (1980).
13. Abram, S. E., Reynolds, A. C. & Cusick, J. F. *Anesth. Analg.* **60**, 81-84 (1981).
14. Lewis, J. W., Cannon, J. T. & Liebeskind, J. C. *Science* **208**, 623-625 (1980).
15. Grevert, P. & Goldstein, A. *Science* **199**, 1093-1095 (1978).
16. Dingle, R., Iversen, L. L. & Breuer, E. *Eur. J. Pharmacol.* **47**, 19-27 (1978).

Nerve growth factor induces adrenergic neuronal differentiation in F9 teratocarcinoma cells

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Teratocarcinoma cells have been used as a model to study differentiation and development in vertebrates¹⁻³. Treatment with retinoic acid (RA) and dibutyryl cyclic AMP can in some embryonal carcinoma (EC) cell lines lead to neural differentiation, as judged by neurofilament expression^{4,5} and by the induction of enzymes involved in cholinergic transmission^{6,7}. Short-term culture of F9 line cells with RA and dibutyryl cyclic AMP results in a biochemically demonstrable rise in acetylcholinesterase (AChE) activity⁸. We now report that

long-term culture of F9 cells with RA and dibutyl cyclic AMP induces neurofilament expression, demonstrated by immunofluorescence with specific antibodies. Furthermore, if nerve growth factor (NGF)⁹ is also added, the developing neurone-like cells exhibit immunoreactivity to tyrosine hydroxylase, a rate-limiting enzyme of catecholamine synthesis specific for adrenergic neurones¹⁰. Immunoreactivity for Leu-enkephalin-like peptides is also induced. These results suggest that F9 cells can differentiate into cells with adrenergic characteristics.

F9 line embryonal carcinoma cells were cultured as monolayers in Eagle's minimum essential medium supplemented with 10% fetal calf serum (FCS)¹¹. For the induction of differentiation, cells were seeded on gelatin-coated glass coverslips at low density (3×10^5 per 50-mm dish) and cultured in 3% FCS in the presence of 1×10^{-7} M RA (Sigma) and 1×10^{-3} dibutyl cyclic AMP (Sigma), added, respectively, on days 2 and 3 of culture. In some experiments, 1 IU ml⁻¹ of NGF was added to the RA- and dibutyl cyclic AMP-containing medium on day 3. The culture medium was changed every 3 or 4 days and the cells were cultured for 21 days. For immunohistochemistry, cells were placed on coverslips and fixed for 30 min with 3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. AChE activity was detected by the Karnovsky-Roots method¹². Immunostaining for tyrosine hydroxylase, neurofilament proteins, Leu- and Met-enkephalins, substance P and glial fibrillary acidic protein was performed by indirect immunofluorescence as described previously¹³ or as indicated in the figure legends.

Neurone-like cells appeared within 7 days in cultures treated with RA and dibutyl cyclic AMP. However, the time needed for differentiation varied between different batches of culture. In these early cultures (7 days *in vitro*) no neurofilament proteins could be demonstrated whereas distinct AChE activity was seen (Fig. 1a). After 14 days *in vitro* the F9 cells were positive on immunofluorescence staining for neurofilaments (Fig. 1b). These cells exhibited no immunoreactivity for tyrosine hydroxylase (Fig. 2a) or for any of the neuropeptides investigated (substance P, Leu- and Met-enkephalin).

When NGF was included in the RA- and dibutyl cyclic AMP-containing culture medium, the neurone-like cells formed extensive processes and became positive for neurofilament proteins within 10 days. All the neurone-like cells in such cultures exhibited immunofluorescence for tyrosine hydroxylase after 11 days *in vitro*, reaching a maximum by 17 days (Fig. 2b). In identically treated cultures some cells showed Leu-enkephalin-like immunofluorescence (Fig. 2c). However, neither substance

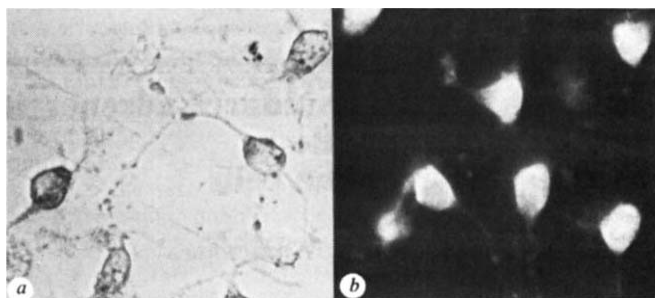


Fig. 1 a, AChE activity in induced (RA and dibutyl cyclic AMP) F9 cells after 7 days *in vitro*. The thiocholine method of Karnovsky and Roots¹² was applied using 1×10^{-5} M tetraisopropylpyrophosphoramide (Light & Co., Colnbrook, UK) as an inhibitor for nonspecific cholinesterases. The reaction product indicating AChE activity can be seen in cytoplasm and in cell processes. $\times 300$. b, Neurofilament immunoreactivity in induced F9 cells after 18 days in culture. Immunostaining for neurofilament triplet proteins was as follows: Rabbit NF antibodies were diluted 1:100 and applied for 1 h at room temperature. The cells were rinsed in phosphate-buffered saline and incubated with sheep anti-rabbit immunoglobulins (Wellcome), diluted 1:20, for 30 min at room temperature. The specificity of the NF antisera used had been confirmed previously^{23,24}. NF immunoreactivity was seen in bi- and multipolar neurone-like cells (arrows). $\times 300$.

P nor Met-enkephalin-like immunofluorescence could be demonstrated. The addition of NGF alone or in combination with only RA or dibutyl cyclic AMP did not lead to neurone-like differentiation. No expression of glial fibrillary acidic protein immunoreactivity was detected in any of the cultures.

In the present study, long-term cultivation (>10 days) of F9 cells was essential for expression of specific neuronal characteristics such as immunoreactivity for neurofilament triplet proteins. NGF was not needed for the induction of neurofilament immunoreactivity, but proved to be critical for the expression of tyrosine hydroxylase, an enzyme specific for catecholamine neurones¹⁰. NGF is essential for the maintenance and development of sympathetic and sensory neurones *in vivo* and *in vitro*^{9,14}, and has been shown to increase the levels of substance P in sensory ganglia of the chick^{15,16} and cholinergic properties in rat cholinergic sympathetic neurones¹⁷. The present results suggest that treatment with RA and dibutyl cyclic AMP is a prerequisite for the ability of NGF to induce the differentiation of F9 cells in an adrenergic direction. This hypothesis is consistent with the suggestion that RA or dibutyl cyclic AMP direct cultured sympathetic neurones to develop in an adrenergic direction, and that the role of NGF might be to enhance the expression of this phenotype to a detectable level¹⁸. NGF was also necessary for the appearance of Leu-enkephalin-like

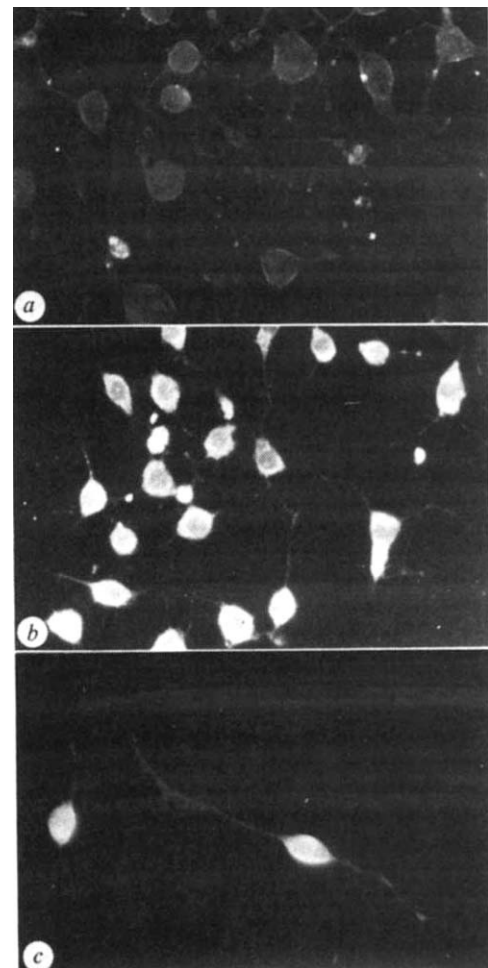


Fig. 2 a, Induced F9 cells immunostained for tyrosine hydroxylase after 17 days *in vitro*. No immunoreactivity can be seen. The specificity of the antibodies had been confirmed previously^{25,26}. $\times 350$. b, Tyrosine hydroxylase immunoreactivity in induced, NGF-treated F9 cells after 17 days in culture. Intensive immunoreactivity is seen in cell bodies and processes of neurone-like cells. $\times 350$. c, Leu-enkephalin-like immunoreactivity in induced, NGF-treated F9 cells after 21 days *in vitro*. The specificity of the antibodies for Leu- and Met-enkephalin and substance P had been confirmed previously²⁷. $\times 280$.

immunoreactivity, a finding which agrees with earlier results showing that catecholamines co-exist with enkephalins in central and peripheral neurones¹⁹. It remains to be shown that the Leu-enkephalin-immunoreactive F9 cells also exhibit tyrosine hydroxylase immunofluorescence. In addition to cholinergic neurones, AChE activity has been demonstrated in cholinergic and adrenergic sympathetic neurones²⁰. Substance P, on the other hand, has been localized mainly in sensory neurones²¹, although recently it has also been found in some sympathetic neurones²². The absence of substance P-like immunoreactivity

Received 3 July; accepted 31 August 1983.

1. Graham, C. F. in *Concepts in Mammalian Embryogenesis* (ed. Sherman, M. I.) 315-394 (MIT Press, Cambridge, 1977).
2. Jacob, F. *Proc. R. Soc. B* **201**, 241-270 (1978).
3. Martin, G. R. *Science* **209**, 768-776 (1980).
4. Darmon, M., Buc-Caron, M.-H., Paulin, D. & Jacob, F. *EMBO J.* **1**, 901-906 (1982).
5. Paulin, D., Jakob, H., Jacob, F., Weber, K. & Osborn, M. *Differentiation* **22**, 90-99 (1982).
6. Adamson, E. D., Evans, M. J. & Magrane, G. G. *Eur. J. Biochem.* **79**, 607-615 (1977).
7. Pfeiffer, S. E. *et al. J. Cell Biol.* **88**, 57-66 (1981).
8. Kuff, E. L. & Fewell, J. W. *Dev. Biol.* **77**, 103-115 (1980).
9. Levi-Montalcini, R. & Angeletti, P. U. *Physiol. Rev.* **48**, 534-690 (1968).
10. Nagatsu, T., Levitt, M. & Udenfriend, S. *Biochem. biophys. Res. Commun.* **14**, 543-549 (1964).
11. Wartiovaara, J., Leivo, I., Virtanen, I., Vaheri, A. & Graham, C. F. *Nature* **272**, 355-356 (1978).
12. Karnovsky, M. J. & Roots, L. *J. Histochem. Cytochem.* **12**, 219-221 (1964).

in F9 cells suggests, on the above hypothesis, that NGF does not favour sensory line differentiation. The NGF-dependent tyrosine hydroxylase immunoreactivity of the F9 cells demonstrate for the first time that NGF has the ability to induce adrenergic neuronal differentiation.

We thank Professor K. Unsicker for purified NGF, Dr A. Keller for tyrosine hydroxylase antibodies, Dr J. Polak for supplying antibodies for substance P, Leu- and Met-enkephalins and Dr D. Dahl for supplying antibodies for glial fibrillary acidic protein and neurofilament proteins.

13. Liesi, P., Dahl, D. & Vaheri, A. *J. Cell Biol.* **96**, 920-924 (1983).
14. Levi-Montalcini, R. & Booker, B. *Proc. natn. Acad. Sci. U.S.A.* **42**, 384-391 (1960).
15. Schwartz, J. & Costa, E. *Brain Res.* **170**, 198-202 (1979).
16. Black, I. & Kessler, J. in *Neurosection and Brain Peptides* (eds Martin, J. B., Reichlin, S. & Bick, K. L.) 287-297 (Raven, New York, 1981).
17. Thoenen, H. & Barck, Y.-A. *Physiol. Rev.* **60**, 1284-1323 (1980).
18. Walicke, P. A. & Patterson, P. H. *J. Neurosci.* **1**, 333-342 (1981).
19. Hökfelt, T. *et al. Proc. R. Soc. B* **210**, 63-77 (1980).
20. Eränkö, O. A. *Rev. Pharmac.* **7**, 203-222 (1967).
21. Hökfelt, T., Elfvin, L.-G., Schulzberg, M., Goldstein, M. & Nilsson, G. *Brain Res.* **132**, 29-41 (1977).
22. Kessler, J., Adler, J., Bohn, M. & Black, I. *Science* **214**, 335-336 (1981).
23. Dahl, D. & Bignami, A. *J. comp. Neurol.* **76**, 645-658 (1977).
24. Dahl, D. *Biochim. biophys. Acta* **668**, 299-306 (1981).
25. Berod, A., Hartman, B. K., Keller, A., Joh, T. H. & Pujol, J. F. *Brain Res.* **240**, 235-243 (1982).
26. Lamouroux, A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3881-3885 (1981).
27. Wharton, J. *et al. Invest. Cell Path.* **2**, 3-10 (1979).

Glycyl glutamine, an inhibitory neuropeptide derived from β -endorphin

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The primary mechanism of activation of intracellular prohormones seems to involve proteolytic cleavage at sequences of consecutive basic residues¹. Thus, all the known biologically active peptides derived from the prohormone of corticotropin and β -endorphin appear to be excised initially by enzymes with this specificity. The C-terminal peptide, β -endorphin (1-31), is generated by cleavage at a lysyl arginine sequence and an additional cleavage can give rise to the related peptides, β -endorphin (1-27) and β -endorphin (1-26). These derivatives of β -endorphin are released by an endopeptidase that appears to catalyse cleavage on the carboxyl side of paired lysine residues, followed by the action of a carboxypeptidase B-like enzyme (Fig. 1). The β -endorphin fragments, β -endorphin (1-27) and β -endorphin (1-26), have been isolated from porcine²⁻⁴ and bovine pituitary⁵ but the C-terminal dipeptide, glycyl glutamine, has not been reported previously. Here we describe the isolation of glycyl glutamine from porcine pituitary and present evidence for its presence in sheep brain stem. When applied ionophoretically to brain stem neurones in the rat, the dipeptide exhibited an inhibitory action on cell firing.

Glycyl glutamine was extracted from porcine pituitary and identified by ion-exchange chromatography (see Fig. 2 legend). The identity of the isolated dipeptide was confirmed by amino acid analysis, yielding glycine and glutamic acid in a ratio of 1.05:1.

Glycyl glutamine was shown to be present in porcine pituitary in substantial quantity (Table 1). When extracted from dissected regions of the pituitary, the dipeptide was found not only in the pars intermedia and posterior pituitary but also in the anterior

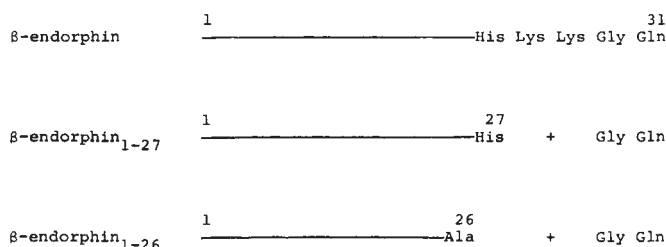


Fig. 1 Diagrammatic representation of β -endorphin and its fragments in pituitary and brain.

pituitary. This was surprising because the 26- and 27-residue forms of β -endorphin occur only to a minor extent in the anterior pituitary and are concentrated in the pars intermedia^{6,7}. It seems likely, therefore, that the glycyl glutamine in the anterior pituitary is derived from the β -endorphin prohormone or its 91-residue fragment, lipotropin, as well as from β -endorphin. In contrast, the glycyl glutamine present in the pars intermedia probably originates directly from β -endorphin, as little lipotropin or prohormone is present in that region.

Further experiments showed that glycyl glutamine occurs in the secretory vesicles of the pituitary. Granules obtained from 1.75 g of porcine pituitary⁸ were lysed by sonication and the dipeptide was extracted and identified by chromatography and amino acid analysis. The results demonstrated that the dipeptide was present in the granule fraction (Table 1), where it seems to be elaborated together with the larger fragments of the ACTH-endorphin prohormone. We therefore conclude that the pituitary granules retain peptides of widely different molecular size, ranging from a dipeptide to a prohormone of 241 residues.

As β -endorphin and α -melanotropin (α -MSH) are known to occur in both NH_2 - and N -acetylated states^{9,10}, experiments were carried out to determine whether glycyl glutamine might be elaborated also in an α , N -acetyl form. The procedure followed the same conditions as those used to identify the non-acetylated form of glycyl glutamine, synthetic α , N -acetylglycyl glutamine (5×10^4 c.p.m., specific activity 20 Ci mmol^{-1}) being used as a marker peptide to guide the isolation of a putative acetylated form of glycyl glutamine. The tritium-labelled dipeptide was not retained during cation exchange chromatography and the excluded fraction obtained was shown by hydrolysis to be devoid of glutamic acid. Thus, the acetylated dipeptide does not exist to a significant extent in porcine pituitary. This indicates that during the processing of the ACTH-endorphin prohormone the enzymes that acetylate β -endorphin and α -MSH exhibit a

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Table 1 Yields of glycyl glutamine obtained from porcine pituitary and ovine brain stem

Source	Tissue weight (g)	Amount of Gly-Gln isolated	% Recovery	Content of Gly-Gln in initial tissue (nmol per g)
Whole pituitary	5.1	98 nmol	21	91.5
Anterior pituitary	6.15	51 nmol	20.4	40.6
Pars intermedia + posterior pituitary	1.5	40 nmol	24	111.1
Pituitary secretory granules	1.75	10 nmol	—	—
Ovine brain stem	34.6	77 pmol	19	11.7

The amounts of the dipeptide were measured on an LKB amino acid analyser Model 4400 and the recoveries were estimated from the quantity of ^3H -labelled dipeptide that appeared in the chromatographed fraction as a percentage of the radioactivity added to the initial homogenate. Because ^3H -glycyl glutamine would not co-centrifuge with the secretory granules, the marker in this case was added immediately before the gel filtration step and no overall yield was calculated.

Fig. 2 Ion exchange chromatography of glycyl glutamine extracted from porcine pituitary. To extract glycyl glutamine from the pituitary, the tissue (5.1 g) was homogenized in 50 ml of acid acetone (acetone, hydrochloric acid, H_2O , 40:1:6) in the presence of a trace amount of ^3H -labelled glycyl glutamine (50,000 c.p.m., specific activity 20 Ci mmol^{-1}). Centrifugation was carried out at 25,000g, 4 °C, for 30 min and after concentration of the supernatant *in vacuo*, gel filtration was carried out on a column (70 × 2 cm) of Sephadex G-75 in 1 M acetic acid. Subsequent ion exchange chromatography of the ^3H -containing fraction was on a column of IR-120 with volatile effluents. ^3H -Glycyl glutamine and ^3H -acetyl glycyl glutamine were added as marker peptides. The column (55 × 0.9 cm) of IR-120 was operated at 50 °C with a linear pH gradient from 0.2 M pyridine formate pH 3.25 to 0.2 M pyridine acetate pH 4.25 (mixer volume 100 ml) and flow rate 1 ml min^{-1} . The peptides in the eluted fractions (2 ml) were located by scintillation counting of aliquots. The material that eluted with the radiolabelled marker was applied to an amino acid analyser and the principal ninhydrin-reactive component was seen to chromatograph in the same position as synthetic glycyl glutamine, immediately after the elution position of valine.

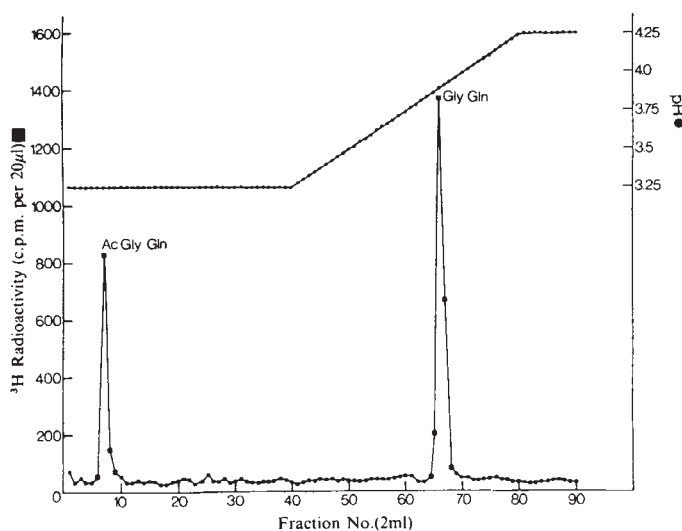
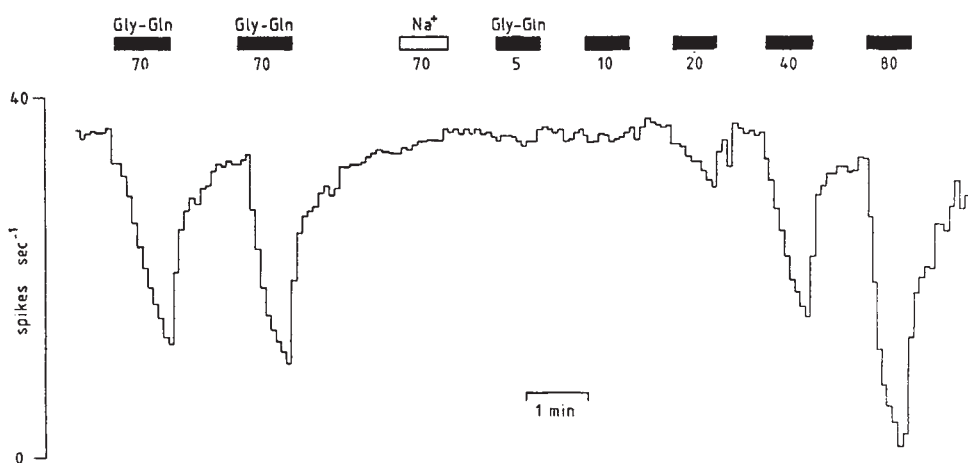


Fig. 3 Inhibition of the firing rate of a neurone in the nucleus reticularis gigantocellularis of rat brain stem by microionophoretic application of glycyl glutamine. Note the slow time course of the inhibitions and their exponential decay. Responses to glycyl glutamine were dose dependent up to 80 nA. Numbers below the bars indicate ionophoretic current in nA and the ordinate represents mean firing rate of the neurone in spikes s^{-1} in successive 5-s periods. Recordings were made through a barrel containing 4 M NaCl, pH 7. Glycyl L-glutamine was ejected as a cation from a barrel with a 50 or 100 mM solution adjusted to pH 3.5–4.5 with 0.5 M HCl. The experiments were performed on adult Sprague–Dawley rats (290–320 g) anaesthetized with urethane (1.5 g per kg intravenously). Single neurones in the brain stem were recorded extracellularly and drugs, including glycyl L-glutamine, were applied via five- or seven-barrelled glass micropipettes (tip diameter 5–8 μm)¹³. Drug ejections were routinely compared with current controls and were occasionally current compensated. Drug-induced excitations or inhibitions were considered significant when they exceeded 30% elevation or reduction in neuronal firing rate respectively. The locations of cells responsive to glycyl glutamine, or at least one cell per brain penetration, were marked by the ejection of Pontamine sky blue dye (100 $\mu\text{A min}^{-1}$) from one barrel of the micropipette followed by histological analysis of 50- μm frozen sections counterstained with Neutral red.



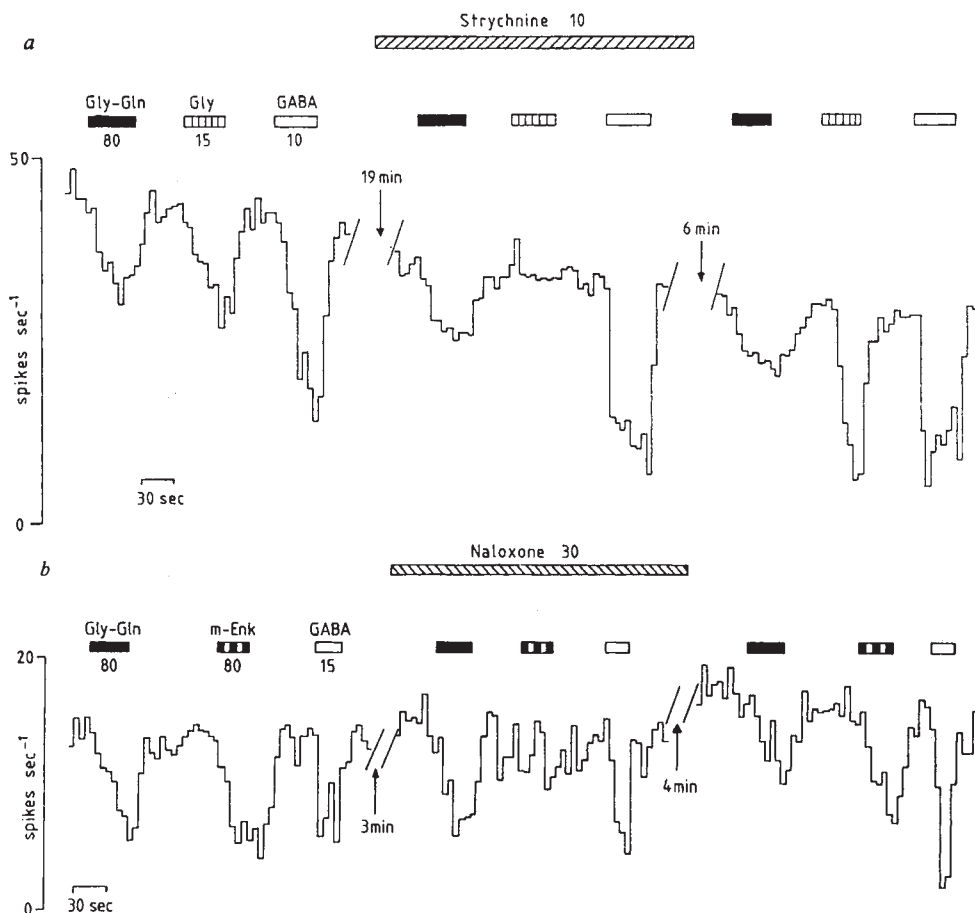
degree of specificity; they do not appear to act on the C-terminal dipeptide.

The formation of glycyl glutamine seems to involve proteolysis at the carboxyl side of the lysyl lysine residues at positions 28 and 29 of β -endorphin, as cleavage on the NH_2 side of these residues would release a tetrapeptide, Lys-Lys-Gly-Gln (Fig. 1). In the present experiments, glycyl glutamine was obtained from the pars intermedia in similar yield to the shortened forms

of β -endorphin, which indicates that the four residues at the C-terminus of β -endorphin are unlikely to be excised as an intact tetrapeptide. Consequently, the synthetic peptide Lys-Lys-Gly-Glu, which has been reported to potentiate the melanotropic effect of α -MSH on anolis lizard skin¹¹, is unlikely to represent a natural fragment of β -endorphin.

It is well established that β -endorphin and its derivatives occur in brain as well as in pituitary, and it was therefore of

Fig. 4 *a*, Absence of antagonism by strychnine of responses to glycyl glutamine (■) and GABA (□). Glycine responses were reversibly antagonized (▨). *b*, Absence of antagonism of glycyl glutamine responses by naloxone (▨). Inhibitory responses to [Met]enkephalin were seen in 8 out of 14 reticular neurones (57%); submaximal responses were reversibly antagonized. Glycine was ejected as a cation from a barrel containing a 10 mM solution at pH 4.5–5.0; strychnine as a cation from a solution of 10 mM strychnine HCl in 150 mM NaCl at pH 6; GABA as a cation from a 100 nM solution at pH 4.5–5.0, naloxone as an anion from a solution of 27 mM naloxone-HCl (Endo) at pH 4.5. In some experiments L-glutamine was ejected as a cation from a 100 mM solution at pH 7.5–8.0. Other details are given in Fig. 3 legend.



interest to demonstrate the presence of glycyl glutamine in the brain stem, a region where the truncated forms of β -endorphin have been shown to predominate¹². Using the same procedure, the dipeptide was extracted from sheep brain stem (including medulla, pons and mid-brain) and identified by comparison of its chromatographic properties with those of synthetic glycyl glutamine. In addition, the shortened forms of β -endorphin were identified by chromatography and radioimmunoassay. The amounts of glycyl glutamine obtained (Table 1) corresponded to the total amounts of β -endorphin (1–27) and β -endorphin (1–26), consistent with its derivation from β -endorphin.

As glycyl glutamine was found to be present in brain and is generated by a mechanism involving cleavage at paired basic residues, the possibility was considered that the dipeptide might have a central function. Glycyl L-glutamine was therefore synthesized and its actions on brain stem neurones were investigated by microiontophoresis. We found that glycyl glutamine applied with currents of 0–100 nA inhibited the firing of 41 out of 109 neurones located within the nucleus reticularis gigantocellularis and nucleus reticularis pontis caudalis of the brain stem reticular formation. Effects were seen both on spontaneously active cells and on cells induced to fire by ejection of DL-homocysteic acid. Most inhibitions were slow in onset (~45–60 s) and the return to baseline firing rate after termination of the ejection current followed a similar time course (Fig. 3). However, a few responses (~10%) were more rapid, resembling the actions of γ -aminobutyric acid (GABA) and glycine (onset ~10–15 s). Both categories of response were dose dependent (see, for example, Fig. 3), typical minimum effective ejection currents being of the order of 40–60 nA applied for 30–35 s.

The actions of glycyl glutamine are not explained in terms of the actions of the constituent amino acids which could be liberated by proteolysis. Although inhibitory responses to glycine were seen on 19 of 21 cells, ionophoretic ejection of strychnine (5–15 nA), which abolished the responses to glycine, had no effect on submaximal responses to glycyl glutamine (Fig. 4a).

Furthermore, in contrast to the inhibitory responses to glycyl glutamine applied at 40–60 nA, the only response observed with L-glutamine at >80 nA was a weak excitation (~25–30% increase in firing rate) in 3 out of 15 cells.

In view of the relationship between glycyl glutamine and β -endorphin, the possibility was considered that the actions of the dipeptide were related to analgesia. It was observed, however, that glycyl glutamine inhibited a similar proportion of neurones (30–40%) responding to either noxious or non-noxious peripheral stimulation (strong mechanical pinch to skin/hindpaws or light touch/hair displacement, respectively). In addition, the inhibitory actions of glycyl glutamine on cells that responded to low-threshold proprioceptive inputs in the dorsal column nuclei indicated that its actions are not specifically related to pain. Note also that the actions of the dipeptide are not explained in terms of 'opioid-like' effects, as ionophoretic ejection of naloxone (20–40 nA), which abolished the responses to [Met]enkephalin, had no effect on submaximal responses to glycyl glutamine (Fig. 4b).

The results show that glycyl glutamine occurs as a natural peptide which can influence the firing of neurones in the central nervous system. Its actions are not mediated by receptors sensitive to strychnine or naloxone. Therefore, the intracellular proteolysis of β -endorphin which occurs in pituitary and brain does not only lead to attenuation of opioid properties; it appears to generate a new biological activity.

We thank Dr S. D. Lovell for assistance in providing samples of sheep brain. J.R.N. was supported by a grant from the MRC to J. H. Wolstencroft. We deeply regret the death of J. H. Wolstencroft on 2 May 1983.

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- Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J. & Tager, H. S. *Ann. N. Y. Acad. Sci.* **343**, 1–16 (1980).
- Bradbury, A. F., Smyth, D. G. & Snell, C. R. *Ciba Fdn Symp.* **41**, (new ser.) 61–74 (1976).
- Smyth, D. G., Snell, C. R. & Massey, D. E. *Biochem. J.* **175**, 261–270 (1978).
- Smyth, D. G., Smith, C. C. & Zakarian, S. in *Advances in Endogenous and Exogenous Opioids* (ed. Takagi, H.) 145–148 (Kodansha/Elsevier, Tokyo–Amsterdam, 1981).

5. Massey, D. E. & Smyth, D. G. *Biochem. Soc. Trans.* **8**, 751-752 (1980).
6. Smyth, D. G. & Zakarian, S. *Nature* **288**, 613-615 (1980).
7. Smyth, D. G., Zakarian, S., Deakin, J. F. W. & Massey, D. E. *Ciba Fdn Symp.* **81**, 79-96 (1981).
8. Jacobs, L. S., McKeel, D. W., Jarrett, I. & Daughaday, W. H. *Endocrinology* **92**, 477-486 (1973).
9. Smyth, D. G., Massey, D. E., Zakarian, S. & Finnie, M. D. A. *Nature* **279**, 252-254 (1979).
10. Harris, J. J. & Lerner, A. B. *Nature* **179**, 1346-1347 (1957).
11. Carter, S. J., Shuster, S. & Morley, J. *Nature* **279**, 74-75 (1979).
12. Zakarian, S. & Smyth, D. G. *Nature* **296**, 250-252 (1982).
13. Normanton, J. R., West, D. C. & Wolstencroft, J. R. *Br. J. Pharmac.* **77**, 477-485 (1982).

Demonstration of B-cell maturation in X-linked immunodeficient mice by simultaneous three-colour immunofluorescence

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CBA/N mice carrying the X-linked immune deficiency gene (*xid*) have fewer splenic B cells than normal CBA mice and are unresponsive to a certain class of antigens¹. Studies of B-cell surface-marker expression² and immune responsiveness³ have led to the commonly accepted idea that the B cells in adult *xid* mice are immature and resemble the B cells of young (1-3 week old) normal mice. That is, like young animals, *xid* mice lack cells in the most numerous of three IgM/IgD B-cell subpopulations (designated I in Fig. 1a, b) present in adult spleen^{4,5}. We now report, however, that this picture is an oversimplification and that in fact the B cells in adult *xid* mice differ from those present in either adult or young normal mice. Using quantitative three-colour fluorescence-activated cell sorter (FACS) analyses, we have compared the correlated expression of IgM, IgD and a newly discovered B-lymphocyte antigen (BLA-1) on splenic B cells in normal and *xid* mice. We show here (1) that most B cells in adult *xid* mice (as in normals) are BLA-1⁻ whereas all B cells in young animals are BLA-1⁺; (2) that the major difference in the IgM/IgD B-cell subpopulations found between *xid* and normal mice is limited to the BLA-1⁻ cells; and (3) that *xid* mice have increased numbers of BLA-1⁺ population III B cells.

All IgM-bearing cells in the spleens of 2-week-old animals express BLA-1 (recognized by rat monoclonal antibody 53-10.1)⁶, whereas less than half of the IgM-bearing spleen cells in adults carry this antigen (Fig. 2). BLA-1 therefore appears to be absent from the late-developing more 'mature' population of B cells that normally predominates in adult animals. The BLA-1 antigen is also found on IgM⁻ cells, particularly in the spleens of young mice (Fig. 2) and in adult bone marrow (data not shown); however, it is absent from Thy-1⁺ cells in spleen or thymus (data not shown) and thus among lymphocytes is expressed only on a subpopulation of B cells (in preparation).

The decreased expression of BLA-1 on 'mature' B cells introduces a new approach for testing the validity of the current paradigm of B-cell development: that the B cells in CBA/N mice are a 'less mature' population of normal B cells and that the inability of these cells to respond to certain antigens is a result of this 'immaturity'³. B cells in adult CBA/N mice have been considered to be less mature because they lack a family of serologically defined B-cell antigens (Lyb-3, 5, 7) which are missing from all B cells in normal neonatal animals, but are present on most B cells in the normal adult⁷⁻⁹. If it is correct that 'mature' B cells fail to develop in *xid* mice, then all B cells in adult *xid* mice should express BLA-1 since this antigen is a marker of immature B cells. We find this not to be the case.

Two-colour analyses of normal and *xid* spleen cells for the correlated expression of IgM and BLA-1 show that whereas all splenic B cells in young normal and *xid* mice bear BLA-1, a

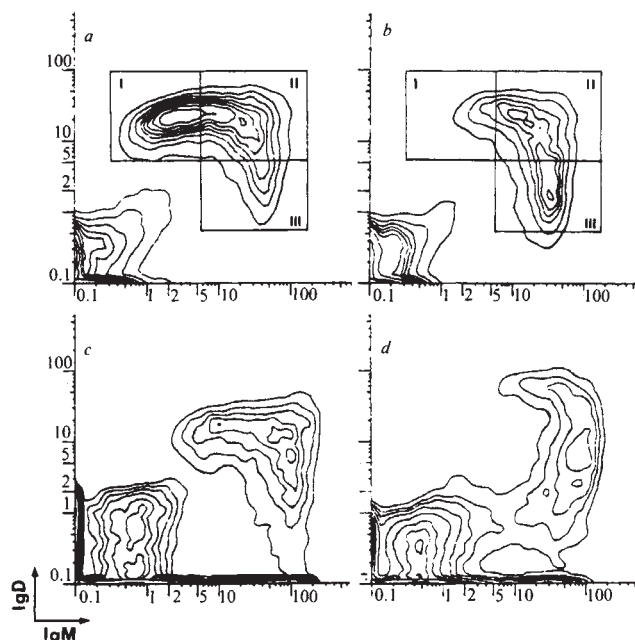


Fig. 1 CBA/N mice lack a population of B cells characterized by high levels of IgD and intermediate to low levels of IgM (two-colour analyses). IgM/IgD-stained spleen cells are shown from: a, CBA, 3 months old; b, CBA/N, 3 months old; c, CBA, 2 weeks old; and d, CBA/N, 2 weeks old. The boundaries of the three previously described B-cell populations^{4,5} are drawn on the contour plots.

Methods: Spleen cells from which the erythrocytes had been lysed by 0.165 M ammonium chloride were stained in a two-step protocol in microtitre wells as described previously¹⁴. Green fluorescence: fluorescein-conjugated rat monoclonal anti-IgM¹⁹ in the first incubation. Red fluorescence: biotinylated mouse monoclonal anti-IgD (specific for the *a* allotype)²⁰ in the first incubation followed by Texas-red-labelled avidin in the second incubation. Stained cells were analysed on a dual-laser fluorescence activated cell sorter FACS equipped with logarithmic amplifiers (for the fluorescence channels) to measure light scatter (size) and the amounts of sets of fluorochrome-labelled monoclonal reagents bound to individual cells¹². Individual measurements on 30,000 (live) cells were collected and stored as list mode data on a VAX-11/780 computer for later analysis. Data are presented as contour plots that can be viewed as representations of three-dimensional surfaces in which the levels of green and red fluorescence per cell define the location of cells on a (64×64) grid and the frequency of cells at each location defines the elevation at that location. After smoothing this surface, contour lines are drawn such that equal numbers of cells (typically 10% of the total) fall between each pair of adjacent contour lines. Regions with more contours thus have greater numbers of cells.

considerable portion of B cells in adult spleens from both types of mouse do not express this antigen (Table 1). Thus, rather than splenic B cells of adult *xid* mice resembling B cells from young *xid* (or normal) mice, they in fact (on the basis of BLA-1 expression) are more similar to normal adult B cells even though they lack the major population of late-arising low-IgM high-IgD B cells present in normal mice (population I).

To resolve the apparent contradiction between the similarity of B cells in normal and *xid* adult animals (with respect to BLA-1 expression) and the previous demonstration that the presumably mature B cells in population I are missing from *xid* spleen, we have developed three-colour immunofluorescence techniques which allow measurement of the correlated cellular expression of BLA-1, IgM and IgD. Previous work¹⁰ described the use of a dye protein (called phycoerythrin, PE) derived from certain strains of algae to obtain good-quality two-colour immunofluorescence from a single laser exciting the fluorescence of fluorescein and PE at 488 nm. Electronic compensation¹¹ for the small overlaps of fluorescein fluorescence on the PE detector

Fig. 2 All IgM⁺ cells in the spleens of young animals are BLA-1⁺ while only a portion of IgM⁺ spleen cells in adults are BLA-1⁺ (two-colour analyses). Spleen cells were from: CBA, 2 week old (*a*) and 2 month old (*b*) mice. See Table 1 for numerical data.

Methods: Spleen-cell suspensions were treated and stained as described in Fig. 1 legend. Green fluorescence: fluorescein-labelled rat monoclonal anti-BLA-1 antibody⁶. Red fluorescence: biotin-labelled rat monoclonal anti-IgM followed by Texas-red-labelled avidin.

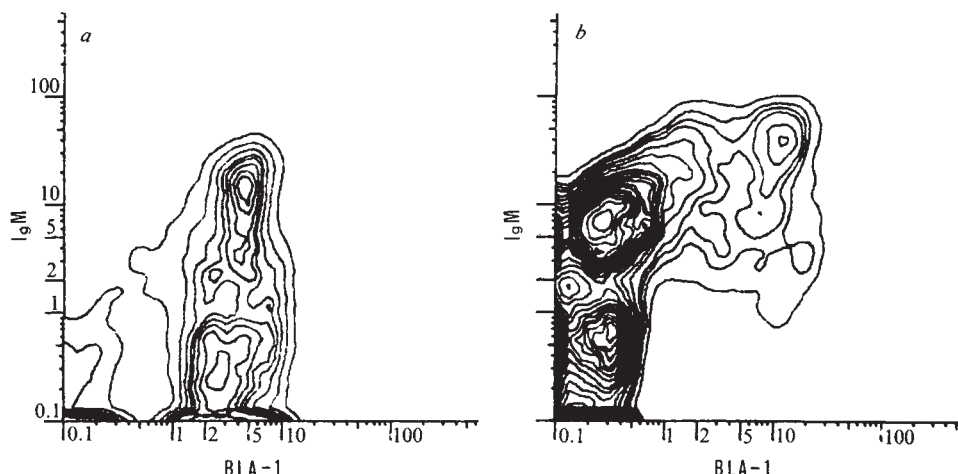


Table 1 Expression of BLA-1 on B cells decreases from 100% in 2-week-old mice to <50% in adults

Strain	Age	IgM ⁺ /BLA-1 ⁻	IgM ⁺ /BLA-1 ⁺
CBA	2 weeks	<1	25
CBA/N	2 weeks	<1	20
CBA	2 months	20	15
CBA/N	2 months	15	10

Cells were stained and analysed as described in Fig. 2 legend. Values report per cent of total spleen cells in each population.

and PE fluorescence on the fluorescein detector yield signals essentially identical to those with either reagent used alone. We have modified our standard two-laser/two-colour setup¹² to allow use of the single-laser/two-colour system in conjunction with the second laser (retuned to 615 nm) to excite a third immunofluorescence from the dye protein, allophycocyanin (APC). This system will be described in detail elsewhere (in preparation).

As expected from two-colour analyses with IgM and BLA-1, three-colour studies with IgM, IgD and BLA-1 show that all splenic B (IgM-bearing) cells are BLA-1 positive in both normal and *xid* animals at 2 weeks of age. Also, as our two-colour studies showed, the correlated expression of IgM and IgD in the two types of mouse at this age is comparable. Surprisingly, we find in analyses of spleen cells from adult animals (Fig. 3) that BLA-1-positive cells have a similar IgM/IgD pattern in both normal and *xid* mice, and that the major difference in the IgM/IgD pattern between normal and *xid* B cells^{4,5,13} is limited to the BLA-1-negative B cells. In CBA (normal) mice, BLA-1⁻ cells are low-IgM/high-IgD (population I) whereas in CBA/N (*xid*) mice BLA-1⁻ cells have high levels of both IgM and IgD (designated population II in 'normal' strains).

Note that although the IgM/IgD pattern of BLA-1⁺ cells is qualitatively similar for both normal and *xid* splenic B cells, *xid* mice have more cells in population III (high-IgM/low-IgD) than normal mice. In general, cells in population III express low levels of Ly-1^{5,13}, previously considered to be a pan-T-cell antigen; *xid* mice have normal numbers of Ly-1⁺ B cells¹⁴, however, so the increase in population III comes from cells that lack Ly-1. Thus, *xid* B cells are not simply a subset of the B cells found in normal mice. That is, their BLA-1⁻ cells are concentrated in population II rather than in population I (as in normals), and their BLA-1⁺ cells include cells in population III for which we have not yet found a counterpart in normal adult mice.

Our working hypothesis is that BLA-1-positive B cells are the precursors of BLA-1-negative B cells. Thus splenic B cells from young animals consist mostly of immature BLA-1-positive B cells whereas spleen cells from adult animals include these cells (characterized by a very reproducible IgM/IgD pattern)

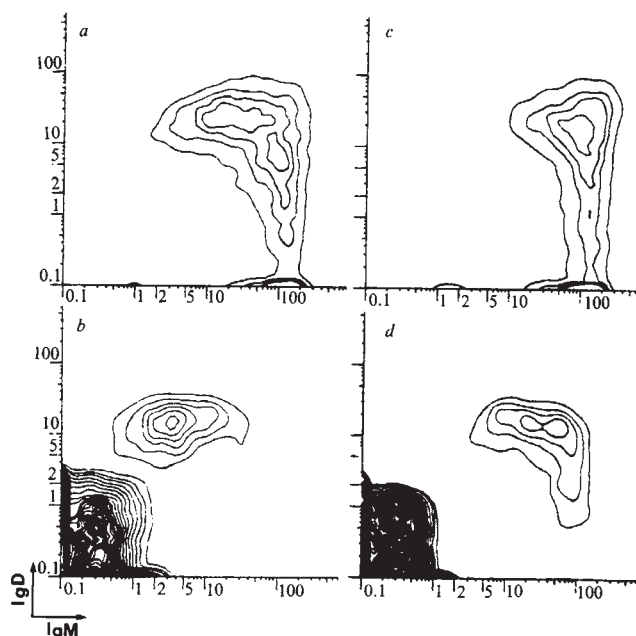


Fig. 3 IgM/IgD patterns for BLA-1⁺ cells in adult spleen are similar for both normal and *xid* B cells, but very different for BLA-1⁻ cells. IgM/IgD contour plots are shown for: *a*, CBA BLA-1⁺ cells; *b*, CBA BLA-1⁻ cells; *c*, CBA/N BLA-1⁺ cells; *d*, CBA/N BLA-1⁻ cells.

Methods: Three-colour immunofluorescence staining procedures were similar to the two-colour procedures described in Fig. 1 legend. Green fluorescence: fluoresceinated anti-IgM in the first incubation. Orange fluorescence: biotinylated anti-IgD in the first incubation followed by PE-labelled avidin in the second incubation. Red fluorescence: APC-conjugated anti-BLA-1 antibody in the first incubation. The PE-avidin conjugate was prepared by cross-linking the two proteins with *N*-succinimidyl 3-(2-pyridyldithio)propionate²¹ generously provided by Mr J. Kimura (B.D. Monoclonal Center, California). APC, a gift of Dr A. N. Glazer (University of California, Berkeley), was cross-linked to the 53-10.1 immunoglobulin⁶ by first labelling the dye protein with succinimidyl 4-(*p*-maleimidophenyl)butyrate²² and then reacting it with mildly reduced (using 20 mM dithioerythritol) 10.1 antibody. Molecular-weight analysis by HPLC (TSK-250 column, Bio-Rad) showed that the conjugate consists primarily of one dye protein coupled to a single IgG. Stained cells were analysed on a dual-laser FACS modified to detect and process the extra fluorescence signal. Three-colour staining data is analysed by taking 'slices' of the histogram generated from one stain (for example BLA-1⁺ cells) and examining the correlated expression of the two other markers (for example IgM and IgD) in this gated population. Such slices are displayed as two-colour contour plots.

and, in addition, have the more mature BLA-1-negative cells (characterized by a distinctive IgM/IgD pattern). We suggest that these immature BLA-1-positive B cells may be the easily tolerized cell type previously described in young animals¹⁵⁻¹⁷. If B-cell tolerization plays an important part in self-nonresponsiveness, then since B cells are continuously being derived from stem cells, there must be a considerable fraction of 'tolerizable' B cells even in the adult animal and perhaps these are the BLA-1-bearing B cells detectable in the adult spleen. Regardless of whether this hypothesis is correct, our data on B-cell subpopulations have important implications for functional studies comparing B cells in normal and *xid* mice.

Many functional studies¹⁸ examining B-cell development and responses rest on comparisons of results obtained using normal B cells (Lyb-3, 5, 7 positive and negative) with those obtained using *xid* B cells (Lyb-3, 5, 7 negative). This assumes that all B cells in *xid* mice are equivalent to the Lyb-3, 5, 7-negative

subpopulation of B cells found in young or adult normal animals. We have now shown, however, that B cells from adult CBA/N (*xid*) mice are not equivalent to immature normal B cells and cannot simply be regarded as Lyb-3, 5, 7-negative cells from normal mice. Specifically, CBA/N mice have 'aberrant' BLA-1⁺ cells (high-IgM/high-IgD) and further BLA-1⁺ cells (high-IgM/low-IgD/Ly-1⁻). Thus we suggest that great caution should be used in drawing conclusions from any study that substitutes CBA/N B cells for immature normal B cells or for normal Lyb-5⁻ B cells.

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1. Scher, I., Frantz, M. M. & Steinberg, A. D. *J. Immun.* **110**, 1396-1401 (1973).
2. Scher, I., Sharrow, S. O. & Paul, W. E. *J. exp. Med.* **144**, 507-518 (1977).
3. Mosier, D. E. *et al. Immun. Rev.* **37**, 89-104 (1977).
4. Hardy, R. R., Hayakawa, K., Haaijman, J. & Herzenberg, L. A. *Nature* **297**, 589-591 (1982).
5. Hardy, R. R., Hayakawa, K., Haaijman, J. & Herzenberg, L. A. in *Immunoglobulin D: Structure and Function* (eds Thorbecke, G. J. & Leslie, G. A.) 112-121 (New York Academy of Science, 1982).
6. Ledbetter, J. A. & Herzenberg, L. A. *Immun. Rev.* **47**, 63-90 (1979).
7. Huber, B., Gershon, R. K. & Cantor, H. *J. exp. Med.* **145**, 10-20 (1977).
8. Ahmed, A. *et al. J. exp. Med.* **145**, 101-110 (1977).
9. Subbarao, B. *et al. J. Immun.* **122**, 2279-2285 (1979).
10. Oi, V. T., Glazer, A. N. & Stryer, L. *J. Cell Biol.* **93**, 981-986 (1982).
11. Loken, M. R., Parks, D. R. & Herzenberg, L. A. *J. Histochem. Cytochem.* **25**, 899-907 (1977).
12. Parks, D. R., Hardy, R. R. & Herzenberg, L. A. *Immun. Today* **4**(5), 145-150 (1983).
13. Herzenberg, L. A. *et al. Immun. Rev.* **67**, 5-31 (1982).
14. Hayakawa, K., Hardy, R. R., Parks, D. R. & Herzenberg, L. A. *J. exp. Med.* **157**, 202-218 (1983).
15. Metcalf, E. S. & Klinman, N. R. *J. exp. Med.* **143**, 1327-1340 (1976).
16. Teal, J. M. & Klinman, N. R. *Nature* **288**, 385-387 (1980).
17. Nossal, G. J. V. & Pike, B. L. in *Immunology 80—Progress in Immunology IV* (eds Fougereau, M. & Dausset, J.) 136-152 (Academic, London, 1980).
18. Singer, A. *et al. Immun. Rev.* **64**, 137-160 (1982).
19. Kincade, P. W., Lee, G., Sun, L. & Watanabe, T. *J. immun. Meth.* **42**, 17-26 (1981).
20. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. *Curr. Topics Microbiol. Immun.* **8**, 115-129 (1978).
21. Carlsson, J., Drevin, H. & Axen, R. *Biochem. J.* **173**, 723-737 (1978).
22. Lee, A. C. J., Powell, J. E., Tregear, G. W., Niall, H. D. & Stevens, V. C. *Molec. Immun.* **17**, 749-756 (1980).

Association of platelet-derived growth factor-induced protein with nuclear material

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Platelet-derived growth factor (PDGF) has been proposed to initiate the cell-cycle traverse of density-arrested BALB/c-3T3 cells by rendering quiescent cells 'competent' to respond to 'progression' factors contained in platelet-poor plasma (PPP)¹. PDGF-treated cells remain competent for many hours following PDGF removal; subsequent addition of PPP triggers G₀-G₁ traversal and entry into S phase^{1,2}. Numerous observations suggest that the competent state reflects the existence of a stable PDGF-induced 'second signal' as opposed to a persistent association of PDGF with cells¹⁻³. Several unique proteins have been shown to be synthesized in response to PDGF; of these, the dose-dependent production of 'pI' (molecular weight 29,000) was found to correlate closely with the induction of competence⁴. We report here the further characterization of pI in terms of its time-dependent synthesis, intracellular location and stability. Electrophoretic analysis of nuclear and non-nuclear extracts of PDGF-treated BALB/c-3T3 cells demonstrated that pI is synthesized during the first 6 h of G₀-G₁, is associated with nuclear material and is stable for 9-12 h. These findings are consistent with the proposed role of pI as the cellular mediator of PDGF.

PDGF-induced pI synthesis appears to be a G₀-specific event. Synchronously growing BALB/c-3T3 cells did not produce detectable pI when exposed to PDGF during S, G₂, or the latter half of G₁ (ref. 5). Consistent with these findings, pI synthesis terminated 4-5 h after addition of PDGF to G₀-arrested cells (Fig. 1). Maximal pI synthesis occurred between 2 and 4 h—this

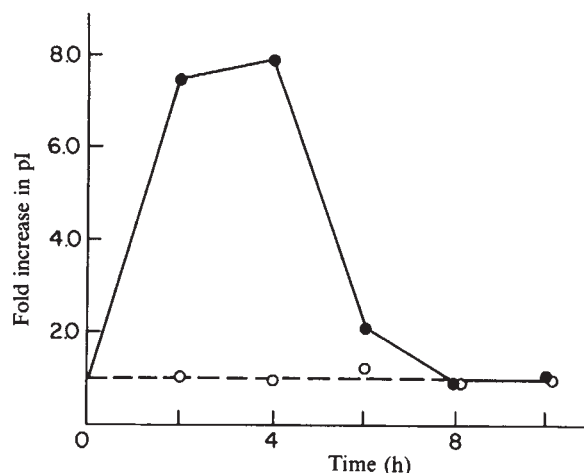


Fig. 1 BALB/c-3T3 cells (clone A31) were grown to confluency in Dulbecco's modified Eagle's medium supplemented with 10% calf serum and used 2-3 days after growth cessation. The cultures in 35-mm culture dishes were refed with 1 ml low-methionine medium containing 10% PPP (○) or 10% PPP with 500 ng ml⁻¹ highly purified PDGF (●). PDGF was prepared from boiled human platelets using CM-Sephadex, Blue-Sepharose and Biogel P150 chromatography^{16,17}. The purity of PDGF used in all experiments was estimated at 25-50 ng per unit, a unit being the amount of PDGF stimulating 50% of the cells in a quiescent confluent BALB/c-3T3 monolayer to synthesize DNA in the presence of 5% PPP. At various times, 30-50 µCi ml⁻¹ ³⁵S-methionine was added; after 20 min, the cells were rinsed, scraped from the plates and resuspended in reticulocyte saline buffer (RSB: 0.01 M NaCl, 0.015 M MgCl₂, 0.01 Tris-HCl, pH 7.4) containing 1% NP40. Following centrifugation at 3,000 r.p.m. for 10 min, the supernatants were heated at 100 °C with 1% SDS and 1% β-mercaptoethanol. Equal amounts of incorporated counts were applied to 6-18% exponential gradients of polyacrylamide¹⁸. Following electrophoresis and fluorographic processing¹⁹, the autoradiograms were scanned on a densitometer. The data are given as fold increase of pI compared with pI at 2 h in PPP-supplemented medium.

Fig. 2 Density-arrested BALB/c-3T3 cells in 100-mm culture dishes were refed with 5 ml medium containing ^{35}S -methionine and either 5% PPP or $1\ \mu\text{g ml}^{-1}$ PDGF. Following a 2.5-h incubation, cells were collected, resuspended in RSB and homogenized. Nuclei were separated from non-nuclear material by low-speed centrifugation. The pelleted nuclei were rinsed three times with RSB and an aliquot solubilized in 1% NP40. The remainder of this crude nuclear material was resuspended in RSB containing 1 M sucrose, overlaid on 1.8 M sucrose and centrifuged at 35,000 r.p.m. for 30 min in an SW50.1 rotor⁶. The pellet of purified nuclei was solubilized in 1% NP40 and samples were electrophoresed on 15% polyacrylamide gels¹⁸. NP40-S, NP40-soluble material; NP40-NS, NP40-insoluble material.

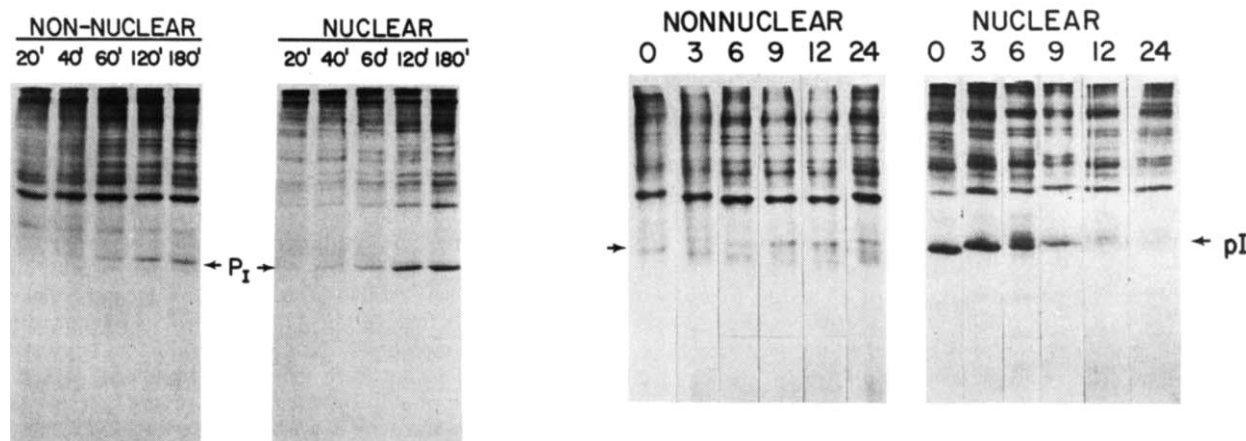
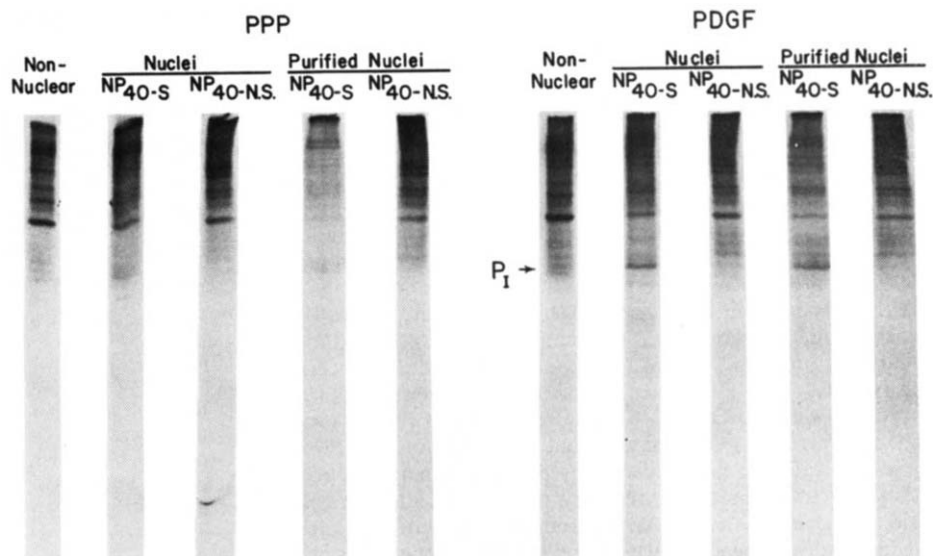


Fig. 3 Confluent BALB/c-3T3 cultures in 100-mm dishes were refed with 5 ml medium supplemented with $1\ \mu\text{g ml}^{-1}$ PDGF and ^{35}S -methionine. At the designated times (in h), non-nuclear and NP40-soluble crude nuclear material were prepared and electrophoresed as described in Fig. 2 legend.

Fig. 4 Density-inhibited BALB/c-3T3 cells in 100-mm dishes were incubated in methionine-free medium containing $1\ \mu\text{g ml}^{-1}$ PDGF and ^{35}S -methionine for 2.5 h. Cultures were rinsed extensively and refed with medium supplemented with 5% PPP and the normal content of methionine. At the indicated times, non-nuclear and NP40-soluble crude nuclear material were prepared and electrophoresed as outlined in Fig. 2 legend.

length of time is sufficient to induce competence in most cells¹. Between 4 and 6 h pI production declined rapidly and by 6–8 h the protein was no longer observable. PPP, which does not initiate cell-cycle traverse, did not stimulate pI synthesis at any of the times tested.

pI was originally observed in Nonidet P-40 (NP40)-soluble extracts of whole cells (see Fig. 1 legend and ref. 4 for procedure), and so was presumed to be a non-nuclear (that is, cytoplasmic or membrane-associated) protein. But when nuclei were first pelleted from homogenates of PDGF-treated ^{35}S -methionine-labelled 3T3 cells and subsequently exposed to NP40, pI could be detected in NP40-soluble crude nuclear material (Fig. 2, lane 7). To ensure that this association was not due to cytoplasmic contamination, crude unsolubilized nuclei isolated from competent cells were further purified by centrifugation through 1.8 M sucrose⁶. pI was also present in the NP40-soluble extract of purified nuclei (Fig. 2, lane 9). Nuclear pelletable material did not contain pI (Fig. 2, lanes 8, 10), nor did any of the fractions prepared from PPP-treated cells (lanes 1–5).

In subsequent experiments, the time-dependent accumulation of pI in nuclear and non-nuclear material was investigated. The amount of pI present in both fractions (that is, the intensity of

the 29,000-molecular weight band) increased in parallel for up to 3 h (Fig. 3). This finding is compatible with the previous demonstration of maximal pI synthesis during the first 4 h of PDGF treatment (Fig. 1) and further shows that nuclear accumulation of pI could be temporally correlated with competence formation. Moreover, pI was detectable in both nuclear and non-nuclear material as early as 40 min after PDGF addition. This rapid induction of pI in response to PDGF has been demonstrated previously in NP40-solubilized whole-cell extracts⁴.

Cell-associated PDGF has a half life of 50–60 min (refs 2, 7). To determine whether pI is more stable in the cell than is bound PDGF, a pulse-chase experiment was performed (see Fig. 4 legend). In both the non-nuclear and NP40-soluble nuclear material, a time-dependent decline in the amount of pI was evident (Fig. 4). pI was especially prominent in the nuclear material at early time points, but by 12 h it was barely visible and by 24 h virtually undetectable. A similar, although less intense, pattern was observed in non-nuclear samples. Figure 4 also shows that the pI localized in nuclear material did not subsequently accumulate in the non-nuclear fraction. Thus, the stability of pI, in both nuclear and non-nuclear material,

approximates the time required for G_1 traverse (12–15 h)¹ and is much greater than that of cellular bound PDGF.

The data presented here and previously suggest that the synthesis of pI signals the growth initiation of density-arrested BALB/c-3T3 cells. As demonstrated by Pledger *et al.*⁴, synthesis of pI can be induced in these cells by competence factors (PDGF and fibroblast growth factor) but not by progression activities (PPP, epidermal growth factor and insulin). Moreover, pI is constitutively synthesized by a spontaneously arising transformed variant (ST3T3) of BALB/c-3T3 which does not require PDGF for growth. We have shown here that the synthesis of pI is rapidly induced by PDGF, peaks at 2–4 h and ceases by 6 h; this temporal sequence is consistent with that required for an early G_1 function. As well as promoting the exit of quiescent cells from G_0 , PDGF has also been shown to prevent the entry of cycling cells into G_0 (ref. 8). The restriction of pI synthesis to density-arrested cells indicates that the effects of PDGF on replicating cells are not mediated by pI; thus pI may function exclusively to facilitate the re-entry of G_0 cells into the cell cycle. It is possible that the synthesis of pI is regulated by later cell-cycle-specific products which prevent pI induction, but not continued cell-cycle traverse, in response to PDGF.

The significance of pI in growth control is further strengthened by the demonstration of its association with purified nuclear material prepared from PDGF-treated 3T3 cells. Centrifugation of nuclei through sucrose has been shown to remove essentially all cytoplasmic contaminants⁹; thus pI presumably is an integral nuclear component of competent cells. Nuclear and non-nuclear 'pI' is assumed to be the same protein on the basis of similar molecular weights on one-dimensional gels and the absence of both from preparations of PPP-treated cells. More rigorous demonstration of identity by two-dimensional gel analysis is in progress.

In all experiments performed as described for Fig. 2, the majority of pI was associated with the nuclear fraction. As shown above, pI remains present in nuclei for up to 12 h following the removal of PDGF from cells. It is conceivable, therefore, that the stability of competence is related to the presence of pI in the nucleus. The rapid accumulation of pI in nuclei further suggests a nuclear function as an early event in the initiation of proliferation of quiescent cells.

Specific nuclear proteins capable of binding to DNA have been previously implicated in growth control^{10–15}. Whether pI is also associated with chromatin has yet to be determined; resolution of the subnuclear location of pI will provide a focus for future studies aimed at elucidating the role of pI in the initiation of cellular proliferation.

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1. Pledger, W. J., Stiles, C. D., Antoniades, H. N. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4481–4485 (1977).
2. Singh, J. P., Chaikin, M. A., Pledger, W. J., Scher, C. D. & Stiles, C. D. *J. Cell Biol.* **96**, 1499–1502 (1983).
3. Smith, J. C. & Stiles, C. D. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4363–4367 (1981).
4. Pledger, W. J., Hart, C. A., Locatelli, K. L. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4358–4362 (1981).
5. Pledger, W. J., Howe, P. H. & Leof, E. B. *Ann. N.Y. Acad. Sci.* **397**, 1–10 (1982).
6. Kletzein, R. F. *Biochem. J.* **196**, 853–859 (1981).
7. Heldin, C. H., Wasteson, A. & Westermark, B. *J. biol. Chem.* **257**, 4216–4221 (1981).
8. Scher, C. D., Stone, M. E. & Stiles, C. D. *Nature* **281**, 1065–1072 (1979).
9. Smith, G. J. *Analyt. Biochem.* **111**, 97–104 (1981).
10. Dippold, W. G., Jay, G., Deleo, A. B., Khoury, G. & Old, L. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1695–1699 (1981).
11. Jay, G. *et al. Cold Spring Harb. Symp. quant. Biol.* **44**, 659–664 (1980).
12. Mercer, W. E., Nelson, D., Deleo, A. B., Old, L. J. & Baserga, R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6309–6312 (1982).
13. Azizkhan, J. C. & Klagsburn, M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2762–2766 (1980).
14. Sen, A. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1647–1651 (1981).
15. Luka, J., Jorvall, H. & Klein, G. *J. Virol.* **35**, 592–602 (1980).
16. Antoniades, H. N. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7314–7317 (1981).
17. Heldin, C.-H., Westermark, B. & Wasteson, A. *Biochem. J.* **193**, 907–913 (1981).
18. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
19. Bonner, W. J. & Laskey, R. A. *Eur. J. Biochem.* **46**, 1279–1283 (1974).

Identification of nuclear proteins encoded by viral and cellular *myc* oncogenes

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The myelocytomatosis viruses are a family of replication-defective avian retroviruses that cause a variety of tumours in chickens and transform both fibroblasts and macrophages in culture through the activity of their oncogene *v-myc*¹. A closely related gene (*c-myc*)^{2,3} is found in vertebrate animals and is thought to be the progenitor of *v-myc*^{4,5}. Changes in the expression and perhaps the structure of *c-myc* have been implicated in the genesis of avian^{6,7}, murine^{8–10} and human^{11–14} tumours (for a review, see ref. 15). Elucidation of the mechanisms by which *v-myc* and *c-myc* might elicit tumorigenesis requires identification of the proteins encoded by these genes. To this end, we have expressed a portion of *v-myc* in a bacterial host and used the resulting protein to raise antisera that react with *myc* proteins. We report here that *v-myc* and *c-myc* encode closely related proteins with molecular weights (MWs) of ~58,000. Integration of retroviral DNA near or within *c-myc* in avian lymphomas^{6,7} apparently enhances expression of the gene^{6,7}. Here we have used cells from one such tumour to identify the protein encoded by *c-myc* and find that the coding domain for the gene is probably intact.

Our strategy for preparing antisera against *myc*-encoded proteins followed that described previously for identifying products of other oncogenes^{16,17}. A portion of the *v-myc* gene was expressed in *Escherichia coli* as a fusion protein with the highly expressed carboxy-terminal half of *v-src* from a plasmid vector using the inducible *trp* promoter. A synthetic linker (AATTATGAATTCAT) inserted at the *EcoRI* site following the *trp* promoter provided a translational initiation codon. The newly created *EcoRI* site within the linker was cleaved, the single-stranded region filled-in with Klenow polymerase in the presence of the four deoxynucleotide triphosphates, and ligated to sequences encoding amino acids 264–515 of *v-src* (derived from digestion of pSRCex16 with *SmaI* and *PvuII*¹⁸). A *HincII* fragment encoding amino acids 107–228 of *v-myc*¹⁹ was inserted 3' of the *src* sequence; this extends the reading frame 122 residues. The final six amino acids are contributed by pBR322 sequences (nucleotides 2,068–2,085²⁰) at which point a translational termination codon occurs. Upon induction, bacterial cells synthesized large amounts of a 43,000-MW polypeptide. The protein was isolated from bacterial extracts by electrophoresis in polyacrylamide gels and injected into rabbits for preparation of antisera as described previously^{16,17}.

To identify the product(s) of *v-myc*, we used quail embryo fibroblasts infected with and transformed by two strains of myelocytomatosis virus, MC29 (ref. 21) and OK-10 (ref. 22). The genome of MC29 virus contains *v-myc* fused to a portion of the viral structural gene *gag*^{23,24}. The hybrid gene gives rise to a 110,000-MW protein (p110^{gag-myc})^{23–25} which is translated from a genome-length mRNA^{24,26} (see Fig. 1, upper panel).

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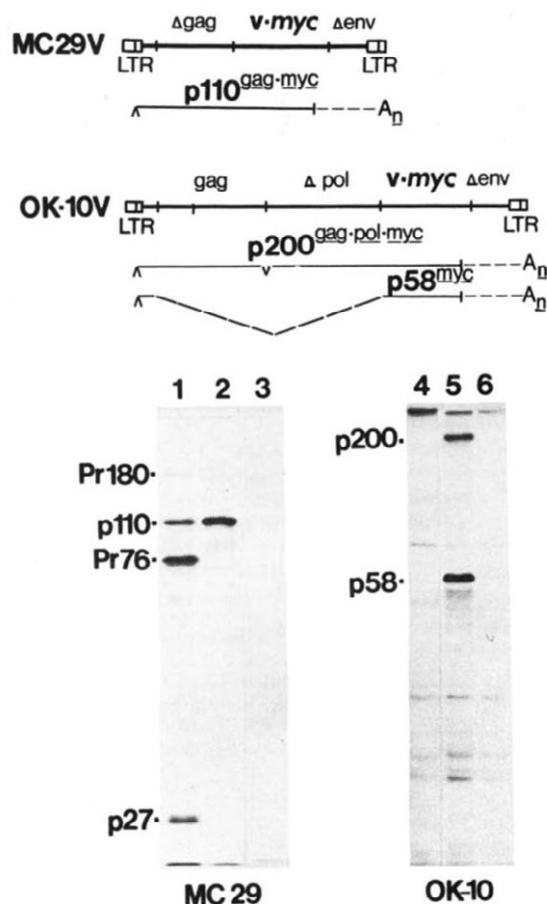


Fig. 1 Immunoprecipitation of myc proteins. The MC-29-labelled gel presents a gel-electrophoretic analysis of immunoprecipitates from ^{35}S -methionine-labelled extracts¹⁷ of Q8 quail infected with MC-29 virus and superinfected with ring-necked pheasant virus. The OK-10-labelled gel shows proteins precipitated from the 9C cell line transformed by OK-10 virus³⁸. The upper part of the figure shows the probable origin of polypeptide p110 from the MC29 virus genome as well as the probable origin of p200 from a genome-length RNA and p58 from a spliced subgenomic RNA of OK-10 virus^{28,29}. The translated portion of RNA is represented by a continuous line. LTR, long terminal repeat of the provirus; A_n, polyadenylation. Pr180, Pr76 and p27 denote structural proteins originating from the genome of ring-necked pheasant virus. Despite the presence of v-src amino acid sequences in the immunogen, precipitation of pp60^{c-src} protein has not been detected with the antisera used here. Apparent molecular weights of polypeptides were interpolated from commercially available standards (Amersham). Lane 1, proteins precipitated with an antiserum against chick retroviral structural proteins; 2, precipitation with 1 μl anti-myc antiserum; 3, 1 μl anti-myc antiserum was blocked by incubation with $\sim 0.05 \mu\text{g}$ of the 43,000-MW protein in a volume of 100 μl for 1 h before immunoprecipitation; 4, normal serum; 5, anti-myc antiserum; 6, blocking experiment, performed as for lane 3.

The genome of OK-10 virus also contains a hybrid gene composed of *gag*, a portion of the gene for reverse transcriptase (*pol*) and *v-myc* (see Fig. 1, upper panel). This gene encodes a 200,000-MW protein (p200^{gag-pol-myc}) as described previously²⁷. In addition, however, *v-myc* of OK-10 virus is expressed independently by translation from a spliced subgenomic mRNA^{28,29} whose protein product may be a 58,000-MW protein identified previously by translation *in vitro*³⁰.

We first tested the reactivity and specificity of our antisera by demonstrating their ability to precipitate p110^{gag-myc} (Fig. 1, lane 2). The precipitation could be blocked by preincubating the antisera with the protein used as the original immunogen

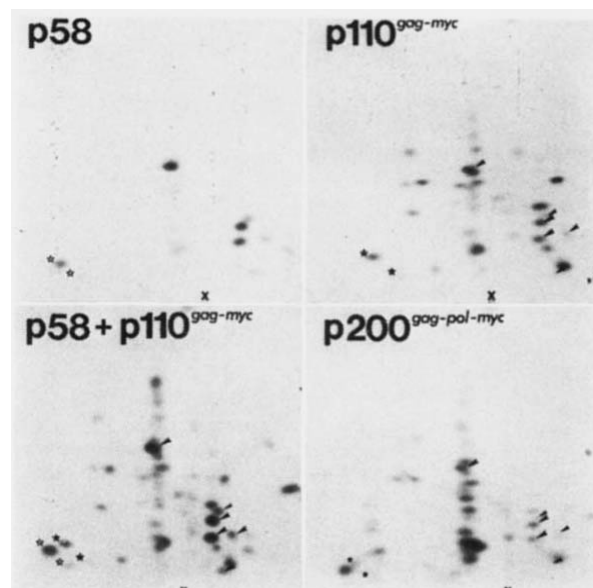


Fig. 2 Two-dimensional peptide mapping of labelled myc proteins after digestion with protease K. Gel slices containing the radiolabelled polypeptides p58, p110^{gag-myc} and p200^{gag-pol-myc} were rehydrated in 300 μl of 50 mM ammonium bicarbonate, pH 8.0, containing 40 $\mu\text{g ml}^{-1}$ protease K (Merck, Darmstadt). After digestion overnight at 37 $^{\circ}\text{C}$, the slices were washed with ammonium bicarbonate and discarded. The combined extracts containing digested and eluted peptides were lyophilized and subjected to two-dimensional analysis by electrophoresis and chromatography on thin-layer cellulose-coated plates³⁹. About 50,000 c.p.m. were applied per plate, resulting in optimal exposures in about 3 days. For comparison of possible co-migrating peptides, samples were mixed in various combinations before analysis. The result of one such mixing experiment between p58 and p110^{gag-myc} peptides is shown. Co-migrating peptides in the two samples are shown by arrowheads, and potentially analogous but differing peptides by stars. X, origin of samples before analysis.

(Fig. 1, lane 3). By contrast, antisera raised against structural proteins of avian leukosis virus (ALV) precipitated both p110^{gag-myc} and viral structural proteins, Pr76^{gag} and p27^{gag} (Fig. 1, lane 1). The precipitations could not be blocked by the myc antigen (data not shown). When cells transformed by OK-10 virus were analysed, the anti-myc sera precipitated p200^{gag-pol-myc} and a 58,000-MW protein (Fig. 1, lane 5). Neither protein was recovered with either preimmune serum (Fig. 1, lane 4) or serum blocked with immunogen (Fig. 1, lane 6). These results support the previous conclusion that p200^{gag-pol-myc} contains a domain encoded by *v-myc* and are consistent with previous suggestions that a 58,000-MW protein (p58^{v-myc-OK-10}) is the product of *v-myc* translated from the subgenomic mRNA of OK-10 virus³¹.

Although the antigen prepared in bacteria contained a sizeable portion of the protein encoded by *v-src*, we were surprised to find that the antisera we raised with this antigen failed to precipitate either the viral or cellular forms of src protein under circumstances where both proteins could be easily detected with authentic anti-src antisera. Although we cannot explain the absence of antibodies directed against src proteins, it simplifies the use of the antisera to study proteins encoded by *myc*.

We confirmed the identity of p58^{v-myc-OK-10} by comparing its two-dimensional peptide map with that of p200^{gag-pol-myc}. Six p58-derived peptides could be identified in protease K digests of p110^{gag-myc} and p200^{gag-pol-myc} (Fig. 2, arrowheads). The remaining two of the eight major p58 peptides (stars in Fig. 2) did not co-migrate with those of p110^{gag-myc} in electrophoresis and chromatography. This could be explained by mutational differences in the *v-myc* sequences of OK-10 and MC29 viruses, or by post-translational modifications in the peptides.

According to previous reports, p110^{gag-myc} is located in the nucleus of MC29V-transformed cells^{32,33}. This result was

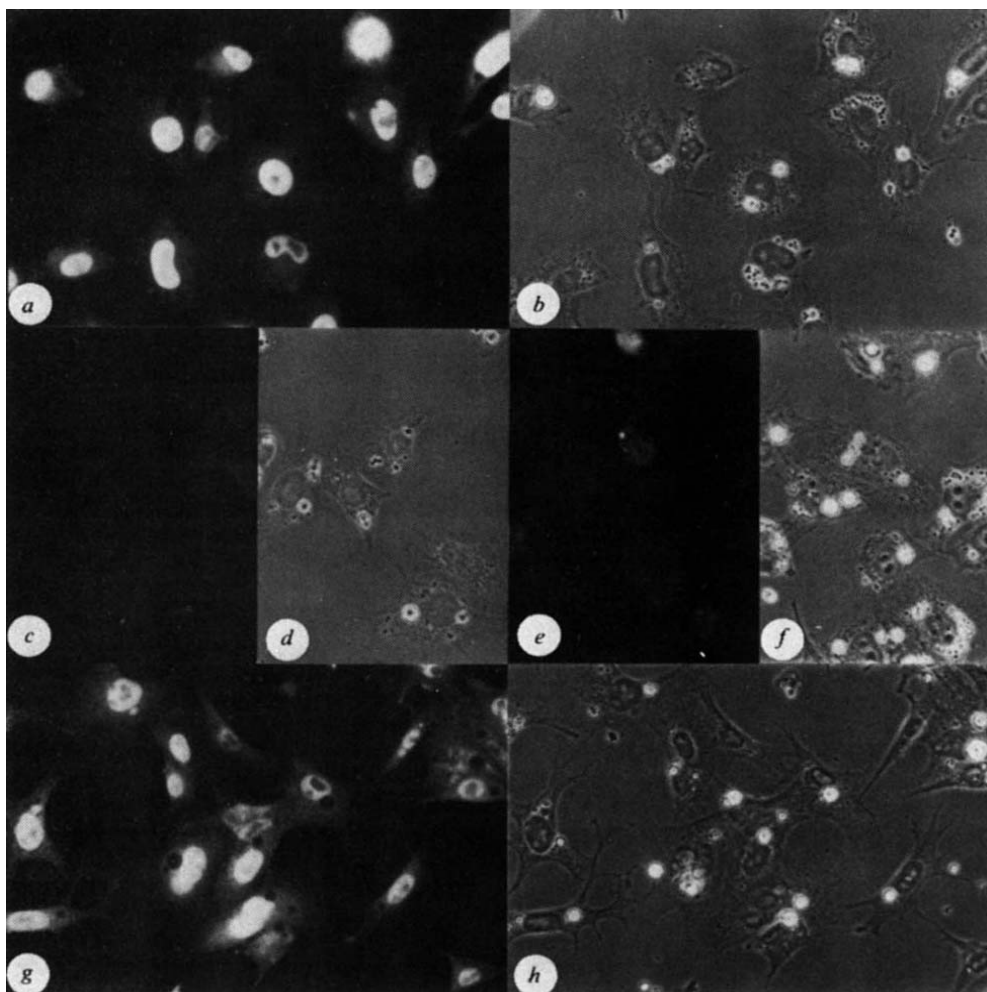


Fig. 3 Localization of myc proteins in myelocytomatosis virus-transformed quail cells. The MC29V- and OK-10V-transformed quail cells used for immunoprecipitation in Fig. 1 were grown on glass coverslips, fixed with 3% paraformaldehyde, permeabilized with NP40 detergent and used for indirect immunofluorescent staining with anti-myc antiserum and rhodamine isothiocyanate-conjugated goat anti-rabbit IgG. *a*, MC29V-transformed Q8 cells stained with anti-myc antiserum diluted 1:50 in phosphate-buffered saline. *b*, Phase-contrast of *a*. *c*, Staining with preimmune serum. *d*, Phase-contrast of *c*. *e*, Cells stained with antigen-blocked antiserum (see Fig. 1 legend). *f*, Phase-contrast of *e*. *g*, OK-10V-transformed cells stained as in *a*. *h*, Phase-contrast of *g*.

obtained with antisera reactive against the *gag*-encoded portion of p110^{gag-myc} polypeptide, using cells which contained p110^{gag-myc} as the only viral protein. We confirmed this finding by immunofluorescence using the anti-myc antisera and extended the observation to OK-10 virus-transformed cells, where proteins p200^{gag-pol-myc} and p58^{myc} would presumably be stained. Figure 3*a* shows myc antigen in MC29V-transformed quail fibroblasts. Lack of staining with preimmune serum (*c*) and with serum preincubated with the immunogen (*e*) demonstrates that the observed nuclear staining is not due to autoantibody in the serum. OK-10V-transformed cells show a similar nuclear staining (*g*). In mitotic cells, myc staining was not associated with chromosomes, but was distributed throughout the cell (data not shown)—an interesting finding in light of the recent claim that myc proteins are associated with chromatin.

The expression of *c-myc* in most normal cells is relatively low³⁴. To detect a protein encoded by *c-myc* more easily, we analysed a cell line (R2B) established from a chicken bursal lymphoma induced by infection with ALV³⁵. Expression of *c-myc* in these cells is enhanced 50–100-fold apparently as a result of integration of ALV DNA in the vicinity of *c-myc*^{6,7}. Analysis of extracts of R2B cells with the anti-myc antisera revealed two proteins (MWs 55,000 and 58,000) whose precipitation could be blocked by competition with the immunogen (Fig. 4*a*, lanes 1, 2). Mapping of the 58,000-MW protein by partial hydrolysis with V8 protease of *Staphylococcus aureus* revealed close similarity to p58^{v-myc} (Fig. 4*b*). We conclude that the 58,000-MW protein is encoded by *c-myc* in the lymphoma cells and have denoted it p58^{c-myc}. Moreover, subcellular fractionation indicates that p58^{c-myc} of R2B cells is also located in the nucleus (G.R. *et al.*, unpublished data). By contrast, maps of the 55,000-MW protein failed to detect any significant relationship to p58^{v-myc} (data not shown). Precipitation of the

55,000-MW protein might occur because the protein binds to p58^{c-myc}. This is unlikely, however, because we have not observed a stoichiometric relationship between the amounts of p58^{c-myc} and p55 recovered from different cell types (Fig. 4, lane 3). We therefore attribute immunoprecipitation of the 55,000-MW protein to cross-reactivities in the antisera.

It is possible that the enhanced expression of *c-myc* in the R2B cells might be accompanied by damage to the coding unit of the gene. We therefore sought another source in which the myc protein would be sufficiently abundant to facilitate unambiguous identification. Previous studies of transcription from *c-myc* have shown that chick erythroblasts transformed by avian erythroblastosis virus (AEV) contain more than average quantities of *c-myc* RNA³⁴, and there is no reason to suspect that *c-myc* has been disturbed or damaged in these cells. Analysis of the erythroblasts with anti-myc antisera revealed relatively small quantities of a 58,000-MW protein, whose immunoprecipitation could be reduced by competition with immunogen (Fig. 4*a*, lanes 3, 4), and whose similarity to p58^{v-myc} could be demonstrated by partial hydrolysis with V8 protease (Fig. 4*b*).

We conclude that v-myc of OK-10 virus and *c-myc* of both lymphoma and erythroleukaemia cells encode a 58,000-MW protein that appears to be located in the nucleus. Similar findings have recently been reported by Hann and colleagues³⁶. Although the proviruses in the lymphoma cells we used (line R2B) have not been studied in great detail, it seems that viral DNA has been integrated in the vicinity of *c-myc* (G. Payne, unpublished data) and that the expression of *c-myc* is enhanced (L. Chen, unpublished data; results presented here). Since the size of the *c-myc* protein in these cells seems normal (by comparison with the protein in erythroleukaemia cells), the coding unit of the gene may not have been damaged by integration of

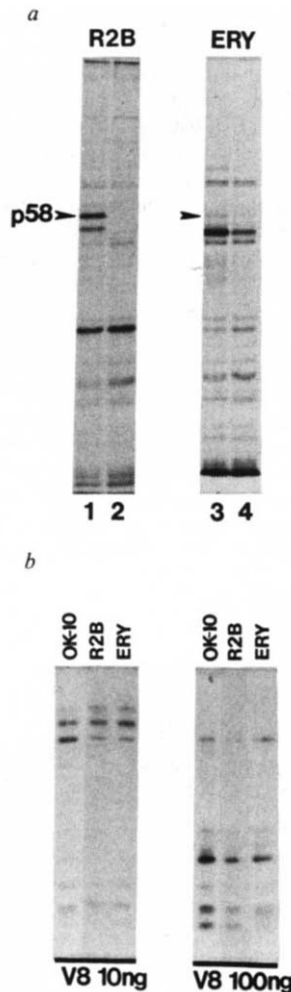


Fig. 4 Immunoprecipitation and analysis of c-myc proteins. *a*: lane 1, polypeptides precipitated from R2B lymphoma cells; lane 2, blocking experiment, performed as Fig. 1; lane 3, polypeptides precipitated from erythroblasts (ERY); lane 4, blocking experiment. *b*, Proteolytic cleavage maps of p58 proteins from OK-10-virus-transformed quail cells, R2B lymphoma cells and erythroblasts. The polypeptide bands were adjusted to similar radioactivity intensities in a preparative polyacrylamide gel, cut out and analysed by proteolytic cleavage with staphylococcal protease V8 (10 ng and 100 ng per lane) in a 16% SDS-polyacrylamide gel.

viral DNA; enhanced expression of *c-myc* may suffice to initiate tumorigenesis by ALV. Analysis of further cell lines and tumours will be required to determine whether this is generally the case, or whether integration of ALV DNA might on occasion perturb the reading frame of *c-myc* and thus change the structure of the protein encoded by the gene.

The apparent size of p58^{v-myc} and its cellular homologue was unexpected, since the nucleotide sequences of *v-myc* and *c-myc* suggest that the open reading frames of these genes may encode a protein of only 47,000 MW^{19,37}. There are at least two possible explanations for this discrepancy: post-translational modification or other structural features of the protein might cause anomalous migration during electrophoresis; or the available nucleotide sequences may not include the entire coding domain of *myc*. Since the *c-myc* protein has the same apparent size in both lymphoma and erythroleukaemia cells, we presume (but cannot yet prove) that p58^{c-myc} represents the complete coding domain of *c-myc*. Identification of the *myc* proteins described here should allow the mechanism of tumorigenesis by *v-myc* and the role of *c-myc* in tumorigenesis to be explored in detail.

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- Beug, H., Hayman, M. J. & Graf, T. *Cancer Surv.* **1**, 205-213 (1983).
- Vennstrom, B., Sheiness, D., Zabielski, J. & Bishop, J. M. *J. Virol.* **42**, 773-779 (1982).
- Robins, T., Bister, K., Garon, C., Papas, T. & Duesberg, P. *J. Virol.* **41**, 635-642 (1982).
- Roussel, M. *et al. Nature* **281**, 452-455 (1979).
- Sheiness, D. & Bishop, J. M. *J. Virol.* **31**, 514-521 (1979).
- Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290**, 475-480 (1981).
- Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295**, 209-217 (1982).
- Shen-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443-452 (1982).
- Calame, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6994-6998 (1982).
- Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7837-7841 (1982).
- Dalla-Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7824-7827 (1982).
- Collins, S. & Groudine, M. *Nature* **298**, 679-681 (1982).
- Dalla-Favera, R., Wong-Staal, F. & Gallo, R. C. *Nature* **299**, 661-663 (1982).
- Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. E. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1707-1711 (1983).
- Klein, G. *Cell* **32**, 311-315 (1983).
- Privalsky, M., Sealy, L., Bishop, J. M., McGrath, J. P. & Levinson, A. D. *Cell* **32**, 1257-1267 (1983).
- Klempnauer, K.-H. *et al. Cell* **33**, 345-355 (1983).
- McGrath, J. P. & Levinson, A. D. *Nature* **295**, 423-426 (1982).
- Alitalo, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 100-104 (1983).
- Sutcliffe, J. G. *Cold Spring Harb. Symp. quant. Biol.* **43**, 77-90 (1978).
- Langlois, A. J., Sankaran, S., Hsiung, P.-H. L. & Beard, J. W. *J. Virol.* **1**, 1082-1084 (1967).
- Oker-Blom, N., Hortling, L., Kallio, A., Nurmiaho, E.-L. & Westermarck, H. *J. gen. Virol.* **40**, 623-633 (1978).
- Bister, K., Hayman, M. J. & Vogt, P. K. *Virology* **82**, 441-448 (1977).
- Mellon, P., Pawson, A., Bister, K., Martin, G. S. & Duesberg, P. H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5874-5878 (1978).
- Kitchener, G. & Hayman, M. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1637-1641 (1980).
- Sheiness, D., Vennstrom, B. & Bishop, J. M. *Cell* **23**, 291-300 (1981).
- Ramsay, G. & Hayman, M. J. *Virology* **106**, 71-81 (1980).
- Chiswell, D. J., Ramsay, G. & Hayman, M. J. *J. Virol.* **40**, 301-304 (1981).
- Saule, S. *et al. J. Virol.* **42**, 71-82 (1981).
- Pachl, C., Biegelke, B. & Linial, M. *J. Virol.* **45**, 133-139 (1983).
- Pachl, C., Schubach, W., Eisenman, R. & Linial, M. *Cell* **33**, 335-344 (1983).
- Donner, P., Grieser-Wilke, I. & Moelling, K. *Nature* **296**, 262-266 (1982).
- Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. *Cell* **29**, 427-439 (1982).
- Gonda, T. J., Sheiness, D. K. & Bishop, J. M. *Molec. cell. Biol.* **2**, 617-624 (1982).
- Chen, L., Courtneidge, S. A. & Bishop, J. M. *Molec. cell. Biol.* **3**, 1077-1085 (1983).
- Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. *Cell* (in the press).
- Watson, D. K., Reddy, E. P., Duesberg, P. H. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2146-2150 (1983).
- Pfeifer, S. *et al. J. Virol.* **46**, 347-354 (1983).
- Ramsay, G. & Hayman, M. J. *J. Virol.* **41**, 745-753 (1982).

Translocation of *c-abl* oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia

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The localization of cellular oncogenes near the break points of tumour-specific chromosomal aberrations suggests an involvement of these genes in the generation of neoplasms^{1,2}. Recently, we demonstrated³ the translocation of the human cellular homologue (*c-abl*) of the transforming sequence of Abelson murine leukaemia virus (A-MuLV) from chromosome 9 to the Philadelphia chromosome (Ph¹) in chronic myelocytic leukaemia (CML). In an attempt to investigate the significance of this translocation in the pathogenesis of CML, we have now studied two CML patients with complex translocations, t(9; 11; 22) and t(1; 9; 22), and two CML Ph¹-negative patients with apparently normal karyotypes. In addition to using blot hybridization with human *c-abl* probes and DNA from

rodent:CML cell hybrids as before, we have used *in situ* hybridization of these probes directly to metaphase chromosomes of CML patients. These studies show that the *c-abl* gene is translocated in Ph¹-positive but not in Ph¹-negative CML patients. CML without the Ph¹ chromosome seems to be a distinct entity with a different origin, and this view is supported by clinical observations including correlations which reveal a poorer prognosis^{4,5}.

The Philadelphia chromosome (Ph¹) results from a specific translocation t(9; 22) (q34; q11) (ref. 6) and occurs in ~92% of adult patients with CML. Among these patients, 6–8% show variant cytogenetic forms in which there is either a complex translocation involving chromosome 9, 22 and at least one other chromosome, or a translocation between chromosome 22 and another chromosome without a detectable aberration of chromosome 9 (refs 7, 8). The presence of the Ph¹ chromosome in CML is of prognostic importance, for it indicates a better response to chemotherapy and a significantly longer survival than for Ph¹-negative patients^{4,5,9}. Our previous study using somatic cell hybrids shows that in cases where the classic t(9; 22) translocation is present, a cellular oncogene, *c-abl*, is transferred

from 9q34 to Ph¹ (ref. 3). To investigate if the *c-abl* gene is involved in the variant forms of CML, we have used *in situ* hybridization with two human *c-abl* probes to chromosomes from a patient with complex Philadelphia translocation, t(9; 11; 22), a Ph¹-negative patient and two healthy individuals, respectively. The probes are a 0.6-kilobase (kb) *Bam*HI fragment and a 1.1-kb *Hind*III-*Eco*RI fragment derived from the 5' and 3' sides of the human *c-abl* gene isolated from a human lung carcinoma cosmid library^{10,11}.

The results of these experiments, which were done independently in Glasgow and Rotterdam using slightly different methods, are shown in Tables 1 and 2 and Fig. 1A and B. The *c-abl* probes hybridize to the distal part of 9q34 in normal controls. In the CML patient with the complex translocation, the probes hybridize to the normal chromosome 9 and to the Ph¹ chromosome. In contrast to this, a strong hybridization signal could be detected only on both chromosomes 9 at band q34 from the Ph¹-negative patient. In particular, no significant hybridization occurred with chromosome 22 and the only other chromosome with more than the expected hybridization is the Y chromosome (Table 1). (The latter result is only evident in

Table 1 Results of *in situ* hybridization to *c-abl* probes

Case (No. of cells analysed)		Normal male (26)			CML Ph ¹ -negative (73)			CML Ph ¹ -positive t(9; 11; 22) (66)		
Chromosome	Relative length (%) [*]	Observed	Expected	χ^2	Observed	Expected	χ^2	Observed	Expected	χ^2
1	8.47	15	16.9	0.21	50	59.2	1.43	57	59.3	0.09
2	7.76	9	15.5	2.73	50	54.2	0.33	40	54.4	3.81
3	6.56	11	13.1	0.34	38	45.8	1.33	40	46.0	0.78
4	6.13	9	12.3	0.88	44	42.8	0.03	41	43.0	0.09
5	5.58	8	11.2	0.91	28	39.0	3.10	34	39.1	0.67
6	5.65	8	11.3	0.96	41	39.5	0.06	36	39.6	0.33
7	5.00	12	10.0	0.40	37	34.9	0.13	40	35.0	0.71
8	4.77	9	9.5	0.03	31	33.3	0.16	27	33.4	1.23
9	4.73	21	9.5	13.92	54	33.0	13.36	28	16.6	7.83
10	4.35	5	8.7	0.92	39	30.4	2.43	31	30.5	0.01
11	4.35	9	8.7	1.57	22	30.4	2.32	13	15.2	0.32
12	4.16	10	8.3	0.35	30	29.1	0.03	30	29.1	0.03
13	3.59	6	7.2	0.20	27	25.1	0.14	25	25.2	0.00
14	3.28	11	6.6	2.93	25	22.9	0.19	28	23.0	1.09
15	3.11	8	6.2	0.52	32	21.7	4.89	20	21.8	0.15
16	3.11	5	6.2	0.23	18	21.7	0.63	24	21.8	0.22
17	3.02	8	6.0	0.67	8	21.1	8.13	20	21.2	0.07
18	2.73	7	5.5	0.41	14	19.1	1.36	19	19.1	0.00
19	2.58	6	5.2	0.12	27	18.0	4.50	17	18.1	0.07
20	2.31	7	4.6	1.25	13	16.1	0.60	12	16.2	1.09
21	1.69	5	3.4	0.75	7	11.8	1.95	16	11.8	1.49
22	1.90	1	3.8	2.06	15	13.3	0.22	8	6.7	0.25
X	5.14	7	5.1	0.71	22	18.0	0.89	20	18.0	0.22
Y	(2.09)	0	2.1	2.10	16	7.3	10.37	5	7.3	0.72
t(9; 11)	6.70	—	—	—	—	—	—	21	23.5	0.27
t(11; 22)	3.30	—	—	—	—	—	—	16	11.6	1.67
Ph ¹	0.98	—	—	—	—	—	—	22	3.4	101.75
Totals:		197	196.9		688	687.7		690	689.9	
χ^2				35.17			58.58			125.77
				$P=0.05$			$P<0.001$			$P<0.0005$
χ^2 admitting chr. 9 & Ph ¹				21.25			45.22			16.19
				NS			$P<0.005$			NS

The major contribution to the nonrandom distribution can be attributed to counts for chromosome 9 and the Philadelphia chromosome. If these are omitted, there is a substantial reduction in χ^2 in all cases, so that the difference between the observed and expected grain counts is no longer significant for the control and the Ph¹-positive CML. An excess of grains over the Y chromosome is largely responsible for the high P value remaining after omitting the chromosome 9 counts in the Ph¹-negative CML case. NS, not significant.

Methods: Chromosomes obtained from peripheral blood were prepared according to standard techniques, stored at room temperature and used for hybridization within 3 months. Chromosomes were banded by treatment with 1% Lipsoil (LIP Equipment and Services, Shipley, UK), stained with Giemsa and photographed. Before hybridization, slides were treated with 100 $\mu\text{g ml}^{-1}$ RNase (Sigma) in 2×SSC at 37°C for 30 min, rinsed well in 2×SSC and dehydrated in ethanol. Chromosome DNA was then denatured in 60% (v/v) formamide/0.1 mM EDTA 15 mM HEPES pH 7 at 55°C for 15 min, rinsed in 2×SSC and dehydrated. A 1:1 mixture of human 3' 1.1-kb *Hind*III-*Eco*RI and 5' 0.6-kb *Bam*HI *c-abl* plasmid DNA was labelled by nick-translation using ³H per dCTP and ³H-dTTR (NEN) to a specific activity of 1×10⁷ c.p.m. per μg . Probe DNA at a concentration of 10 $\mu\text{g ml}^{-1}$ in 50% (v/v) formamide/0.6 M NaCl/5 mM HEPES pH 7/10 mM EDTA/10% dextran sulphate was hybridized to denatured chromosomes at 43°C for 12 h. Slides were rinsed in 0.1×SSC at 65°C for 30 min followed by extensive washing in 0.1×SSC for 12 h at 4°C, and finally dehydrated in ethanol. Preparations were exposed to Ilford K2 emulsion for 33 days at 4°C. Previously photographed spreads were relocated and scored for silver grains.

* Expressed as a percentage of the X-containing haploid set¹⁷.

Table 2 Analysis of chromosomal distribution of grains on normal lymphocytes and on peripheral blood cells of CML patients

	Chromosome no.	DNA content*	No. of cells	Grains	
				Observed	Expected
Control	9/9	4.74	18	15	3.9
46, XX	22/22	1.72		2	1.4
	All	100		83	
Ph ¹ -neg. CML	9/9	4.74	15	8	3.7
46, XY	22/22	1.72		2	1.3
	All	100		78	
CML	9	2.37	12	7	1.9
46, XY, t(9; 11; 22)	9q+	3.32		3	2.6
(q34; q12; q11)	11/11q-	4.04		3	3.2
	22	0.86		1	0.7
	22q-	0.52		7	0.4
	All	100		79	

Grains were counted on complete, well spread metaphases. The background level of grains on the cells was always less than two-thirds of the grains counted on the genome. Distribution of grains is uniform and random on all chromosomes (data not shown) except the specific signal on chromosomes 9 and 22q-.

Methods: Chromosomes were prepared as described in Table 1 legend. They were not identified before hybridization. Following RNase treatment, chromosomes were denatured in 70% formamide/2×SSC at 70°C for 2 min, rinsed three times in 2×SSC and dehydrated in ethanol. The same probe DNAs as in Table 1 were labelled by nick-translation using ³H-dCTP and ³H-dTTP (NEN) to a specific activity of 1.2×10⁸ c.p.m. per µg. The probes were denatured for 5 min at 70°C at a concentration of 0.2 µg ml⁻¹ in 50% (v/v) formamide/2×SSC (0.3 M NaCl/0.03 M trisodium citrate/0.04 M NaPO₄, pH 6)/10% dextran sulphate combined with a 1,000-fold excess of alkali-denatured salmon sperm DNA carrier added to the chromosomes and hybridized for 10 h at 37°C. Slides were rinsed in three changes of 50% formamide 12×SSC at 39°C for 15 min each, followed by five changes in 2×SSC at 39°C and 5 h washing in 2×SSC at 4°C. After dehydration in ethanol, preparations were exposed to Kodak NTB2 emulsion for 10 and 18 days, respectively, developed, and stained with Giemsa. Well spread metaphases were chosen and grains counted. After destaining in methanol/acetic acid (3:1), chromosomes were re-analysed using reverse (R) banding with acridine orange, after heat denaturation. Chromosomes could now be identified individually. A comparison of grains localized before and after R-banding (data not shown) revealed essentially the same results as presented.

*Refs 18–20.

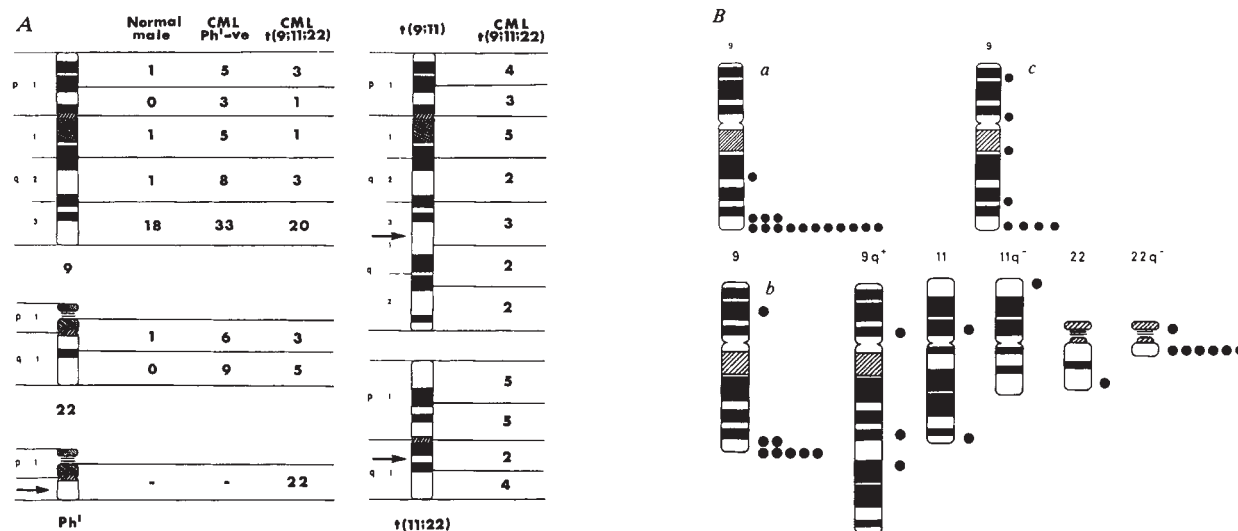


Fig. 1 A, Diagram of the intrachromosomal distribution of total grains (from Table 1) over chromosomes 9, 22 and the translocation chromosomes in a normal control, Ph¹-negative CML patient, and the t(9; 11; 22) CML patient. Each chromosome is divided into sections, each approximately 1% of the total X-containing haploid set, for ease of scoring. The disproportionate number of grains in the distal third of the long arm of chromosome 9 and over the Ph¹ chromosome is evident. There are no significant differences observed over other regions. B, Distribution of silver grains on chromosomes hybridized to *c-abl* probes (see Table 2). A significant ($P > 0.001$) grain accumulation is noted over band q34 of chromosome 9 in a healthy control (a). In a CML patient with a t(9; 11; 22), the hybridization signal ($P < 0.001$) is found both on the normal chromosome 9 and Ph¹ (b), while in the Ph¹-negative CML (c) a grain accumulation is only found on chromosome 9q34 ($P < 0.01$).

the Glasgow data, and the distribution of grains over the Y appears even, in contrast to the results for chromosome 9.)

These results were confirmed by Southern blot analysis of panels of somatic cell hybrids derived from cells of a second CML patient having the complex translocation t(1; 9; 22) and a second Ph¹-negative CML patient. The hybrid cell lines (Table 3) were obtained by fusion of cells from mouse (WEHI-3B) or Chinese hamster (a3 or a23) origin with the patients' leukocytes. Figure 2 shows that the 3' *c-abl* probe hybridizes to DNA from cell line WEDY-9 (Ph¹) but not to DNA from 8CB-7B, 8CB-8A or WEDY-7 (chromosome 9q+). Again, the recipient chromosome for the distal part of chromosome 22, 1p-, does not

hybridize (12CB-17B). Similar results were obtained with the 5' probe (data not shown).

Using a panel of hybrid cell lines containing chromosome 9 or 22 from a patient with Ph¹-negative CML (Table 3), the 3' probe always hybridized to DNA from cell lines containing chromosome 9 (17CB-3B, 17CB-21C, 16CB-16A, 16CB-20) but not to cell lines containing chromosome 22 in the absence of chromosome 9 (16CB-15A, 16CB-17, 17CB-13, 16CB-1). Similar results were obtained with the 5' probe (data not shown). For the Ph¹-negative CML patient, these results could be explained by assuming either that non-leukaemic cells had been involved in the cell fusion, or that only the 'normal' chromosome

Table 3 Human chromosome content of human-mouse and human-Chinese hamster somatic cell hybrids

Hybrid	Human chromosomes																						Fig. 2					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		X	Y	1p--	9q+	22q--
8CB-7B	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-	+	-	g
8CB-8A	+	-	+	+	+	+	-	+	-	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	f
12CB-17B	-	-	-	+	+	-	-	+	-	+	+	-	+	-	+	-	+	-	-	-	-	+	+	-	+	-	-	h
WEDY-7	+	+	-	+	+	+	-	+	-	+	-	+	+	-	-	+	+	-	+	-	-	+	+	-	-	-	+	e
WEDY-9	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-	+	-	+	+	+	-	-	-	+	d
17CB-3B	+	+	-	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	h
17CB-21C	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	j
16CB-16A	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	e
16CB-20	+	+	-	+	-	+	+	+	+	+	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	g
16CB-15A	+	-	+	+	-	+	-	-	-	-	+	-	+	+	-	-	-	+	+	+	+	+	-	-	-	-	-	d
16CB-17	+	-	+	+	-	-	-	+	-	-	-	+	-	+	-	-	+	+	+	+	+	+	-	-	-	-	-	f
17CB-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	i
16CB-1	+	-	+	+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	-	-	-	c

The origin and details of the initial characterization of the somatic cell hybrids obtained by using blood from the CML patient with a t(1;9;22) are described elsewhere^{21,22}. WEDY-7 and WEDY-9 are hybrids obtained from fusion with mouse WEHI-3B cells, while Chinese hamster cell line a3 was used to produce the 8CB-7A and 8CB-8A hybrids. All other hybrid cell lines were obtained from fusion with Chinese hamster a23 cells. Chromosome analysis was done using reverse (R) banding with acridine orange, after heat denaturation, and performed on the same batches of hybrid cells used for DNA isolation. At least 16 metaphases per cell line were analysed. The right-hand column refers to the lanes of the Southern blots shown in Fig. 2A and B.

Fig. 2 Localization of *c-abl* sequences in somatic cell hybrids obtained from cells of two patients suffering from CML, showing: A, a complex type of Philadelphia translocation, t(1;9;22); and B, an apparently normal karyotype. A, Detection of the human 3' end 5.0-kb *EcoRI* *c-abl* fragment in the DNAs from: a, Chinese hamster (a3); b, mouse (WEHI-3B); c, human placenta; d, WEDY-9; e, WEDY-7; f, 8CB-8A; g, 8CB-7B; h, 12CB-17B. B, Detection of the human 3' end 5.0-kb *EcoRI* *c-abl* fragment in DNAs from: a, Human placenta; b, Chinese hamster (a3); c, 16CB-1; d, 16CB-15A; e, 16CB-16A; f, 16CB-17; g, 16CB-20; h, 17CB-3B; i, 17CB-13; j, 17CB-21. For details of cell lines see Table 3 legend.

Methods: All cell lines used in these experiments were grown in large batches (10^7 – 10^8 cells) and DNA was prepared as described by Jeffreys and Flavell²³. *EcoRI*-restricted DNA (10 µg per lane) from human placenta, hybrid cell lines, mouse and hamster fusion partners were electrophoresed on 0.7% agarose gels. *HindIII*- and *HindIII*-*EcoRI*-digested λ DNAs were included as molecular weight markers (not shown). After blotting to nitrocellulose, the filters were hybridized to the 3' end 1.1-kb *HindIII*-*EcoRI* *c-abl* restriction fragment. Hybridization and washing procedures (to $0.1 \times \text{SSC}$ at 65 °C) were carried out according to the method of Bernards and Flavell²⁴.

22 and 9 had been retained in the hybrids. In the absence of genetic markers to distinguish the respective homologues of chromosome 9 and 22, the latter explanation cannot be excluded. It is, however, unlikely, for the following reasons: (1) peripheral leukocytes from the patient contained 98% myeloid cells, and (2) assuming random chromosome segregation in hybrids, it is unlikely that the 'normal' chromosomes 22 or 9 are individually selected.

The mechanisms by which the translocation of oncogenes may be related to malignant transformation remain unknown. It is unlikely to be fortuitous that in Ph^1 -positive CML the *c-abl* gene is transferred from chromosome 9 to a site on chromosome 22 adjacent to the band containing the λ immunoglobulin light-chain gene cluster^{12,13}. The same type of chromosomal rearrangement has been shown to occur in cases of Burkitt lymphoma between the site of an oncogene on chromosome 8 (*c-myc*) and the site of immunoglobulin heavy-chain genes on chromosome 14^{14,15}. In the case of the t(8;22) translocation in Burkitt's lymphoma, there is recent evidence from *in situ* hybridization which could be interpreted as suggesting that the λ immunoglobulin locus is transferred from chromosome 22 to the site of *c-myc* on chromosome 8q24 (ref. 16). The common factor in Ph^1 -positive CML and these other cases could be that the chromosomal rearrangement places the oncogene in a transcriptionally active site^{1,2} where it may initiate neoplastic transformation. The results presented here show that patients with Ph^1 -negative CML have a distinct subclass of myelocytic leukaemia with regard to clinical, cytogenetic and oncogenetic characteristics. In this subclass of CML, neoplastic transformation is likely to have an altogether different origin.

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- Klein, G. *Nature* **294**, 313–318 (1981).
- Rowley, J. D. *Science* **216**, 749–751 (1982).
- de Klein, A. *et al.* *Nature* **300**, 765–767 (1982).
- Ezdinli, E. Z., Sokal, J. E., Crosswhite, B. S. & Sandberg, A. A. *Ann. intern. Med.* **72**, 175–182 (1970).
- Mintz, U., Vardiman, J., Golomb, H. M. & Rowley, J. D. *Cancer* **43**, 411–416 (1979).
- Rowley, J. D. *Nature* **243**, 290–293 (1973).
- Rowley, J. D. *Clin. Haemat.* **9**, 55–86 (1980).
- Sandberg, A. A. *The Chromosomes in Human Cancer and Leukemia* (Elsevier, New York, 1980).
- Sandberg, A. A. *Cancer Genet. Cytogenet.* **1**, 217–228 (1980).
- Heisterkamp, N. *et al.* *Nature* **299**, 747–750 (1982).
- Heisterkamp, N., Groffen, J. & Stephenson, J. R. J. *molec. appl. Genet.* (in the press).
- Erikson, J., Martinis, J. & Croce, C. M. *Nature* **294**, 173–175 (1981).
- McBride, O. W. *et al.* *J. exp. Med.* **155**, 1480–1490 (1982).
- Croce, C. M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 3416–3419 (1979).
- Erikson, J., Ar-Rushdi, A., Drwinga, H. L., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 820–824 (1983).
- De la Chapelle, A. *et al.* *Nucleic Acids Res.* **11**, 1133–1142 (1983).
- Ferguson-Smith, M. A. *La Ricerca Clin. Lab.* **4**, 297–335 (1974).
- Mendelsohn, M. L., Mayall, B. H., Bogart, E., Moore, D. H. II & Perry, B. H. *Science* **179**, 1126–1129 (1973).
- Mayall, B. H., Carrano, A. V., Moore, D. H. II & Rowley, J. D. *Cancer Res.* **37**, 3590–3593 (1977).
- Wayne, A. W. & Sharp, J. C. *Cancer Genet. Cytogenet.* **5**, 253–256 (1982).
- Geurts van Kessel, A. H. M., van Aghoven, A. J., de Groot, P. G. & Hagemeijer, A. *Hum. Genet.* **58**, 162–165 (1981).
- Geurts van Kessel, A. H. M., Tetterton, P. A. T., von dem Borne, A. E. G. K., Hagemeijer, A. & Bootsma, D. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Jeffreys, A. J. & Flavell, R. A. *Cell* **12**, 429–439 (1977).
- Bernards, R. & Flavell, R. A. *Nucleic Acids Res.* **8**, 1421–1533 (1980).

Solvent-induced distortions and the curvature of α -helices

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The α -helix defined in 1951 by Pauling *et al.*¹ on the basis of model building and X-ray fibre diffraction data has 3.65 residues per turn (n) achieved with planar peptides, torsion angles of $\phi = -48^\circ$ and $\psi = -57^\circ$ and hydrogen bonds which are close to linear. Although X-ray analyses of proteins have confirmed the general correctness of the model for the helix, recent high resolution (1.7–1.0 Å) diffraction studies have shown that the parameters described by Pauling *et al.*¹ and later by Perutz² and Arnott and Wonacott³ are not a good description of the α -helices in globular proteins^{4–7}, where the mean values of ϕ , ψ are usually close to -63° , -42° . Here we show that these values arise as a mean of two significantly different classes in amphipathic helices depending on whether the peptide carbonyl oxygen is hydrogen bonded to a solvent or polar side-chain atom. The hydrogen bonds made by the hydrophilic carbonyls to the NH groups within helices are longer and less linear than those involving hydrophobic carbonyls. We also show that these effects are associated with a significant curvature of helices in globular proteins. For example, the α -helix in avian pancreatic peptide (aPP) has a radius of curvature of approximately 70 Å. These results are of significance in the packing of helices in fibrous and globular proteins, in the calculation of their dipole moments, solvent accessibilities and internal energies, and in the theoretical estimation of spectroscopic properties such as circular dichroism and Raman scattering.

Parameters proposed for α -helices, along with those found in highly refined proteins, are summarized in Table 1. Watson¹² and Perutz *et al.*⁸ noted that α -helices in globular proteins are usually amphipathic and that when the main chain is in contact with solvent the carbonyls of the residues appear to point slightly away from the helix axis. Similar observations were later discussed by Blundell and Johnson⁹, by Finney *et al.*¹⁰ and Sakabe *et al.*¹³. Nevertheless, amphipathic helices have been generally

considered to be regular. However, the refinement of avian pancreatic polypeptide (aPP)⁴ at 0.98 Å has provided a precise model for a helix of five turns, and has detailed the peptide hydrogen bonding both within the helix and with side-chains and solvent. Figure 1 shows two typical peptide groups in which it can be clearly seen that the presence of a water molecule causes the peptide group to rotate so that the carbonyl forms a strong hydrogen bond with the water molecule and a hydrogen bond in which $C_iO_iN_{i+4}$ is quite far from linear. Analysis of the Ramachandran plots of ϕ_i and ψ_i for α -helix residues showed no systematic grouping, but a plot of ψ_i , ϕ_{i+1} —the two angles around a peptide between residues i and $i+1$ —shows significant differences depending on whether the peptide carbonyls are hydrogen bonded to groups other than those within the main chain of the helix.

The discovery of these effects encouraged us to make a more systematic survey of six α -helices, of more than two turns in length in the highly refined proteins actinidin⁵, ribonuclease⁷ and aPP⁴. The helices analysed are defined in Fig. 2 which shows a graph of the $C_iO_iN_{i+4}$ angles against the O_i-N_{i+4} distances. Of the 55 hydrogen bonds analysed, 25 involve carbonyls that are in a hydrophilic environment, and are hydrogen bonded to a water molecule or side chain. The mean O_i-N_{i+4} distances

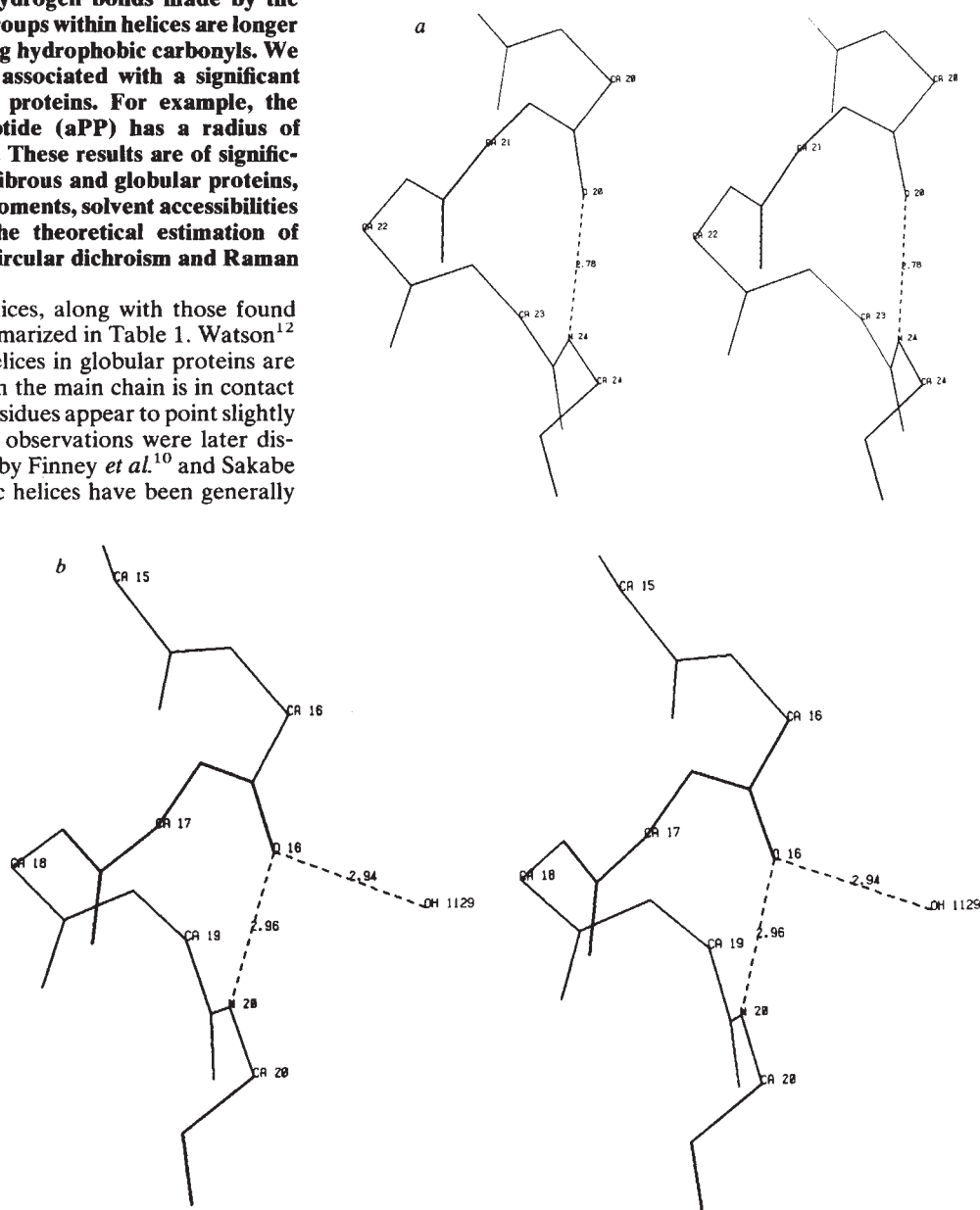


Fig. 1 Stereo-views of helix hydrogen bonds in aPP. The diagrams illustrate the increased $C_iO_iN_{i+4}$ angle and O_iN_{i+4} distance caused because of the presence of a water molecule. Hydrogen bond distances (O_iN_{i+4} , in Å) are indicated in the diagrams. *a* Shows the carbonyl of residue 20, where the oxygen is hydrogen-bonded only to the amide of residue 24. The $C_iO_iN_{i+4}$ angle is 165° . *b* Shows the carbonyl of residue 16, where the oxygen is hydrogen-bonded to the amide of residue 20 and also to a water molecule shown as OH. The $C_iO_iN_{i+4}$ angle is 146° .

for 'hydrophilic' and 'hydrophobic' hydrogen bonds are 3.09 (0.13) and 2.91 (0.06) Å respectively. The corresponding mean $C_i\hat{O}_iN_{i+4}$ angles for the two classes are 148.5 (6.0)° and 159.0 (4.5)°. Analysis of the variances for the samples (using a standard *t*-test for small samples) shows that the differences between the means for both $C_i\hat{O}_iN_{i+4}$ angle and O_i-N_{i+4} distance are significant to better than 0.1%.

Hydrophilic hydrogen bonds are thus longer and less linear than hydrophobic ones. Helix carbonyls forming hydrogen bonds with solvent or polar side-chain atoms become tilted further outward from the helix axis. This causes the $C_i\hat{O}_iN_{i+4}$ angle to decrease and the O_i-N_{i+4} distance to increase.

The additional tilt of these carbonyls is reflected in the torsion angles ψ_i and ϕ_{i+1} as shown in Fig. 3. For hydrophilic carbonyls of residue *i*, ψ_i has a mean value of -41 (6)° whereas the mean value for carbonyls which are not hydrogen bonded to solvent or side chains is -44 (6)°. The corresponding values of ϕ_{i+1} for the two classes are -66 (5)° and -59 (6)°. The means for the two classes are again significantly different to better than 0.1%.

These amphipathic helices also show a general curvature. The α -helix in aPP is shown in Fig. 4a, b and has a radius of curvature of approximately 70 Å. The helix curves so that the centre of curvature is on the hydrophobic side of the helix and roughly on a line defined by the 'hydrophobic moment'¹¹ of the amphipathic helix. For a given helix, the degree of curvature may be expected to depend upon the extent of its amphipathic character. We have generated, using computer graphics a series of helices with different amphipathic characters, using the average parameters of the 'hydrophobic' and 'hydrophilic' parts of known helices as given in Table 1. Figure 4c shows that such helices

Table 1 Parameters of helices

Helix	ϕ (°)	ψ (°)	<i>n</i>	<i>p</i> (Å)	<i>d</i> (Å)	O_i-N_{i+4} (Å)	$C_i\hat{O}_iN_{i+4}$ (°)
Pauling <i>et al.</i> ¹	-48	-57	3.65	5.51	1.51	2.89	165.2
Perutz ²	-67	-44	3.67	5.20	1.42	2.67	144.8
Arnott <i>et al.</i> ³	-57	-47	3.59	5.46	1.52	2.96	156.0
'Hydrophilic' environment	-66	-41	3.62	5.32	1.47	2.88	147.4
'Hydrophobic' environment	-59	-44	3.55	5.47	1.54	3.04	153.2

ϕ and ψ are the main torsion angles around $-C_{i-1}-N_i-C\alpha_i-C_i-$ and $-N_i-C\alpha_i-C_i-N_{i+1}-$, *n* is the number of residues per turn, *p* is the pitch and *d* the rise per residue along the helix axis. The 'hydrophilic' helix is generated by giving all residues the parameters for residues in which the carbonyl is hydrogen bonded to a solvent molecule or side chain, whereas the 'hydrophobic' helix has the parameters of residues whose carbonyls do not form such bonds. The parameters are derived from several highly refined proteins: avian pancreatic polypeptide (aPP)⁴, actinidin⁵ and ribonuclease⁷.

with high hydrophobic moments have the highest curvature, while those in an entirely hydrophobic or entirely solvent surrounded environment are linear. However, a putative hydrophobic helix generated in this way has hydrogen bonds which are surprisingly long at 3.04 Å. The short bonds of the 'hydrophobic' parts of the amphipathic helix are therefore dependent on its curvature and a linear hydrophobic helix would appear to be less stable. This may be one reason why α -helices are rarely found to pass through the hydrophobic core; they are almost always on the surface.

The curvatures for a total of 30 helices (>2 turns in length) have now been analysed, using the method described in the legend to Fig. 4. For 16 of the helices, however, fitting of the appropriate probe helix indicated local distortions in the real helix, which prevented straightforward interpretation of their curvature. Marked 'kinks' were observed in 4 of these helices, where significant distortions occurred at single sites in the middle of their structures. A further 3 were regular in the middle of their length but showed irregularities at the termini and 9 showed irregularities along their entire length.

For the remaining 14 (regular) α -helices, where the calculated curvature is the result of bending rather than 'kinking', 11 were observed to have a radius of curvature in the range 40 to 100 Å

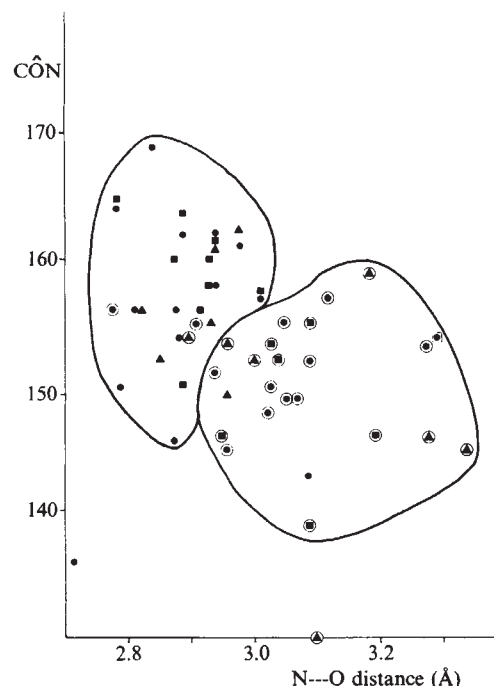


Fig. 2 A graph of the $(C_i\hat{O}_iN_{i+4})$ angles against the O_i-N_{i+4} distances for six helices of the proteins actinidin⁵, shown by ● (helix 1, residues 25–43; helix 2, 71–81; and helix 3, 121–129), ribonuclease⁷, shown by ▲ (helix 1, 3–13; helix 2, 24–33) and avian pancreatic polypeptide (aPP)⁴, shown by ■ (15–32). Where the carbonyls are also hydrogen bonded to a water molecule or an atom of a side chain, the symbols are circled. (The symbol □ indicates a bifurcated hydrogen bond for the carbonyl of residue 73, in helix 2 of actinidin.)

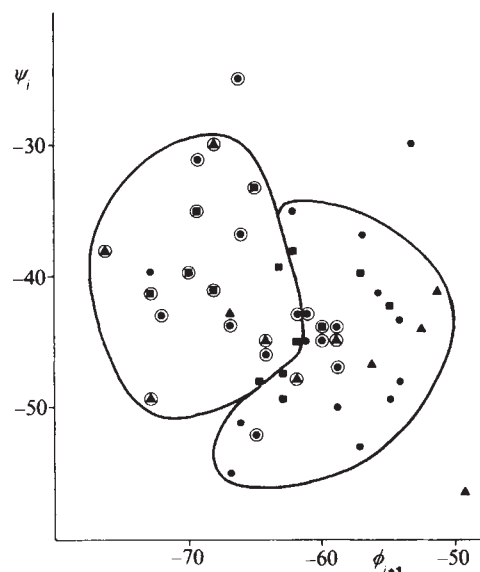
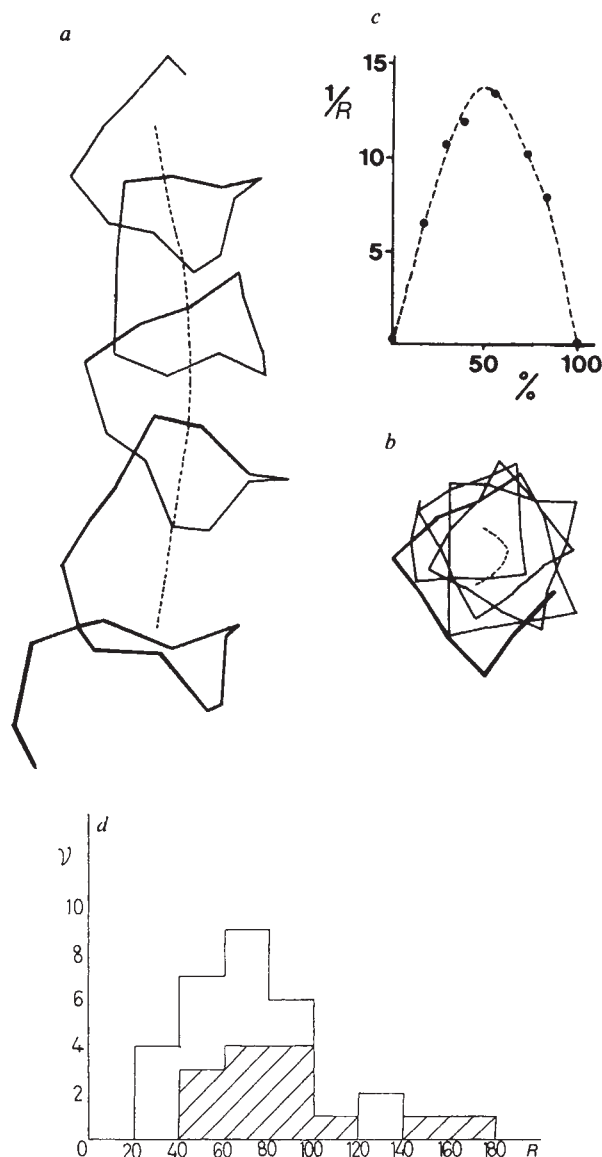


Fig. 3 A graph of the torsion angles ψ_i and ϕ_{i+1} for the six helices defined in the legend to Fig. 2.

and are considered to be curved. Three have a radius of curvature in the range 100 to 180 Å and are essentially linear. For one of these linear helices (involving residues 40–55 in phospholipase A₂) a cystine residue in the centre of the structure is anchored by a disulphide bond to the body of the protein and may prevent the helix from assuming any greater curvature.

A histogram showing the distribution of helix curvatures is illustrated in Fig. 4d. Despite their distortions, most of the non-regular helices have a radius of curvature in the same range as the regular helices. These initial results clearly show that the majority of α -helices are distinctly curved. A detailed discussion of this analysis will be presented elsewhere).

Fig. 4 The curvature of the α -helix of aPP. The helix is viewed perpendicular to the 'axis' (shown as a dashed line) in *a*, and down the 'axis' (with the N-terminal end nearest the observer and tilted slightly downward) in *b*. The 'axis' shown effectively represents the path followed by a probe helix as a moving fit is obtained for this helix along the length of the real helix. The values of ψ , ϕ and ω for the probe helix were taken as the mean values for the real helix analysed. (A correct choice is important in obtaining a smooth curve.) The probe helix was first superimposed upon the real helix to give a least-squares fit for the main-chain atoms 1 to 5 in each helix. Using the transformed coordinates for the entire (5 turn) probe helix the axis for the probe helix was then determined and the coordinates calculated for the projection of the first real helix α -carbon onto this line. This process was then repeated 'moving' the probe helix 'along' the real helix to obtain a least-squares fit for atoms 4 to 8, then 7 to 11 and so on (that is, the process was repeated for each residue in the real helix). The points representing projections of the alpha carbons onto the probe helix axis for each fit, were used to calculate a radius of curvature for the real helix. This was taken to be that of the best circle through the points after their projection into the least-squares plane. *c* Shows a plot of $1/\text{radius of curvature}$ ($1/R \times 10^{-3} \text{ \AA}$) for eight model-built 5-turn helices with differing amphipathic characters (ranging from an entirely 'hydrophobic' helix to an entirely 'hydrophilic' one). The helices were constructed with different percentages of residues forming the 'hydrophobic surface' (as indicated by % on the figure). Residues forming part of this 'hydrophobic surface' were assigned ψ/ϕ of -44° , -59° , and the remainder ψ/ϕ of -41° , -66° . The radii of curvature were calculated as described above. *d* Shows the distribution of curvatures for the 30 α -helices analysed. R is the radius of curvature (in $\text{\AA}$) and ν indicates the number of helices with R in a given range. Bars in outline show the data for all helices analysed; those that are cross-hatched show the data for regular helices only.



What are the consequences of curved amphipathic helices? The curvature may be important in defining the packing of helices, both in globular tertiary structures and in fibrous proteins. For example, the α -helix in aPP curves around the hydrophobic core. If the sequence is such that the hydrophobic residues do not form a simple hydrophobic side to the helix but rather form a helix of longer pitch on the surface, then the helix would have an inherent tendency to supercoil. Thus the individual α -helical strands of α -fibrous proteins, such as myosin, tropomyosin and keratin would be expected to prefer a supercoiled structure independent of any optimisation of packing of side chains.

The parameters defined here for α -helices have important implications for theoretical calculations. Both the tilting out of the peptides and the curvature of the helix will affect estimations of the helix dipole, and of Raman, IR and circular dichroism.

The observations reported here show that water can perturb the secondary structure of a protein locally and that small perturbations may be amplified in a long helix. They show that the α -helix is not regular as has been assumed but rather it is sequence dependent where the helix is involved in tertiary or quaternary interactions. Finally, we may expect similar distortions of other perfect secondary structures; in particular similar effects due to water interactions may accentuate the twist of β -sheets.

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Chothia, Willie Taylor, John Finney and Julia Goodfellow for useful discussions. The experiments were carried out using the SERC computer graphics workstation at Birkbeck. D.J.B. is supported by a SERC Instant award grant, N.B. by a SERC research grant and J.M.T. by a SERC Advanced Fellowship.

Note added in proof: Following discussions with C. Chothia, we would like to emphasize that we do not see the distortions caused by solvent as the sole cause for helix curvature. Further structural details and higher resolution X-ray analyses are required for most of the 30 helices examined here for curvature alone. Packing restraints clearly cause helix kinks and may affect curvature, and systematic variation of peptide bonds from planarity may compensate for distortions induced by solvent or side-chain binding to carbonyls.

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1. Pauling, L., Corey, R. B. & Branson, H. R. *Proc. natn. Acad. Sci. U.S.A.* **37**, 205-211 (1951).
2. Perutz, M. F. *Nature* **167**, 1053-1054 (1951).
3. Arnott, S. & Wonacott, A. J. *J. molec. Biol.* **21**, 371-383 (1966).
4. Glover, I. *et al. Biopolymers* **22**, 293-304 (1983).
5. Baker, E. N. *J. molec. Biol.* **141**, 441-484 (1980).
6. Steigemann, W. & Weber, E. *J. molec. Biol.* **127**, 309-338 (1979).
7. Borkakoti, N. E. *J. Biochem.* **132**, 89-94 (1983).
8. Perutz, M. F., Kendrew, J. C. & Watson, H. C. *J. molec. Biol.* **13**, 669-678 (1965).
9. Blundell, T. L. & Johnson, L. N. *Protein Crystallography* (Academic, New York, 1976).
10. Finney, J. L., Gellatly, B. J., Golton, I. C. & Goodfellow, J. *J. Biophys. Soc.* **32**, 17-33 (1980).
11. Eisenberg, D., Weiss, R. M. & Terwilliger, T. C. *Nature* **299**, 371-379 (1982).
12. Watson, H. C. *Prog. Stereochem.* **4**, 299-333 (1969).
13. Sakabe, N., Sakabe, K. & Sasaki, K. in *Structural Studies on Molecules of Biological Interest* (eds Dodson, G., Glusker, J. P. & Sayre, D.) 509-526 (Clarendon, Oxford, 1981).

Structure and mechanism of copper, zinc superoxide dismutase

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Copper, zinc superoxide dismutase (SOD) catalyses the very rapid two-step dismutation of the toxic superoxide radical (O_2^-) to molecular oxygen and hydrogen peroxide through the alternate reduction and oxidation of the active-site copper¹. We report here that after refitting and further refinement of the previous 2 Å structure of SOD², analysis of the new model and its calculated molecular surface shows an extensive surface topography of sequence-conserved residues stabilized by underlying tight packing and H-bonding. There is a single, highly complementary position for O_2^- to bind to both the Cu(II) and activity-important Arg 141 with correct geometry; two water molecules form a ghost of the superoxide in this position. The geometry and molecular surface of the active site, together with biochemical data, suggest a specific model for the enzyme mechanism.

The two identical subunits of an SOD dimer are related by a non-crystallographic 2-fold axis that orients the two active sites facing away from each other with the Cu atoms 33.8 Å apart. Each 151-residue subunit has an eight-stranded β -barrel of Greek key topology³ plus three external loops of non-repetitive structure. Starting from the previously determined 2 Å crystallographic structure of bovine erythrocyte SOD at 25.5% residual error², we included active-site water molecules, geometrically⁴ added hydrogens to one of the two dimers in the crystallographic asymmetric unit, refitted the model to electron density maps (comparing superimposed subunits) and refined 14 more cycles against both structure factors and stereochemical parameters (Hendrickson-Konnert refinement modified to include H)⁵. The resulting structure⁶ has a residual of 19.3% for 15,253 X-ray diffraction data (which included only the larger peaks between 4 and 2 Å spacing); the associated r.m.s. deviation from ideal bond lengths is 0.029 Å.

Following refinement, molecular surfaces were calculated for each of the four crystallographically independent subunits (two dimers) using Connolly's program MS (refs 7, 8), which represents the solvent-accessible molecular surface of Richards⁹ as a smooth, continuous lattice of points. A colour vector-graphics display of the skeletal model and its associated molecular dot-surface was used to analyse the SOD active-site channel.

The active-site Cu ion is at the bottom of a deep channel on the outside of the β -barrel between two large loops (Fig. 1). The Cu(II) exposes about 5.2 Å² to a 1.4 Å radius probe, but the Zn(II) is completely buried. Metal ligands form the floor of the narrow part of the channel (~3 Å × 15 Å) which lies between Lys 134 and Arg 141. Eighteen solvent-exposed residues, of which 16 are conserved in the four published SOD sequences¹⁰⁻¹³, form the molecular surface of the active-site channel, which has an area of ~660 Å² (11% of the total exposed surface). Of this area, 86% belongs to main-chain or invariant side-chain atoms; by contrast, only 41% of the remaining molecular surface area comes from invariant atoms. This invariance suggests that the topography of the active channel is critical to the enzyme's function.

The surface shows two pits forming specific binding sites in the narrowest part of the active-site channel floor (Fig. 2a, b),

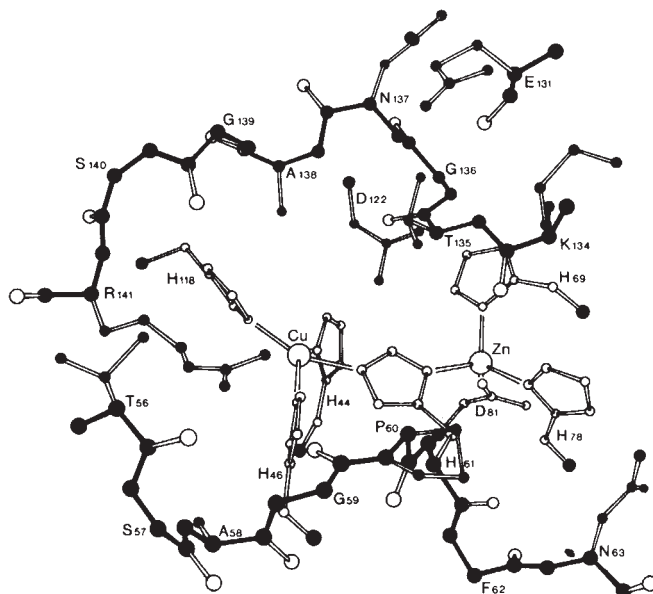


Fig. 1 Drawing from ORTEP plot²⁸ showing the residues of the active-site channel as viewed from the solvent. Main chain is shown black, ligand side chains open, and other side chains with solid atoms and open bonds. Residues 131, 134-139 and 141 form one rim of the channel, residues 56, 58-60 and 63 form the other rim, and the metals and their ligands form the floor. Main-chain atoms only of residues 57, 62 and 140 have also been included in the drawing for continuity. The Cu(II) is liganded by His 118, 44, 61 and 46 (clockwise from upper left) with an uneven tetrahedral distortion from square planar geometry ($\angle 61NE2-Cu-118NE2 = 160^\circ$; $\angle 44ND1-Cu-46NE2 = 130^\circ$). The geometry of Zn ligands His 61, 69, 78 and Asp 81 (clockwise from left) is tetrahedral, with the largest distortion involving His 69 ($\angle 69ND1-Cu-81OD1 = 89^\circ$; $\angle 69ND1-Cu-78ND1 = 119^\circ$). Asp 81 is behind the Zn and is buried. His 61 bridges between the Cu and Zn, with its ring almost planar to the metals but tilted towards the solvent by ~0.3 Å on the Cu side. In the metal-liganding residues, all five of the non-liganding imidazole nitrogens and the non-liganding Asp 81 carboxyl oxygens are H-bonded². Reduction of the Cu during catalysis should weaken the H-bonds of the His rings bound to the Cu (ref. 29), allowing small shifts in ligand geometry that could give a more tetrahedral Cu site. The carboxyl group of solvent-inaccessible Asp 122, which forms a second, less direct bridge between the metals by H-bonding to both Zn ligand and His 69 and Cu ligand His 44, may couple the Cu oxidation state to the Zn geometry: coupled movement of Asp 122 and His 69 away from His 44 and towards His 78 in the Cu(I) enzyme would decrease the distortion in the Zn tetrahedral geometry.

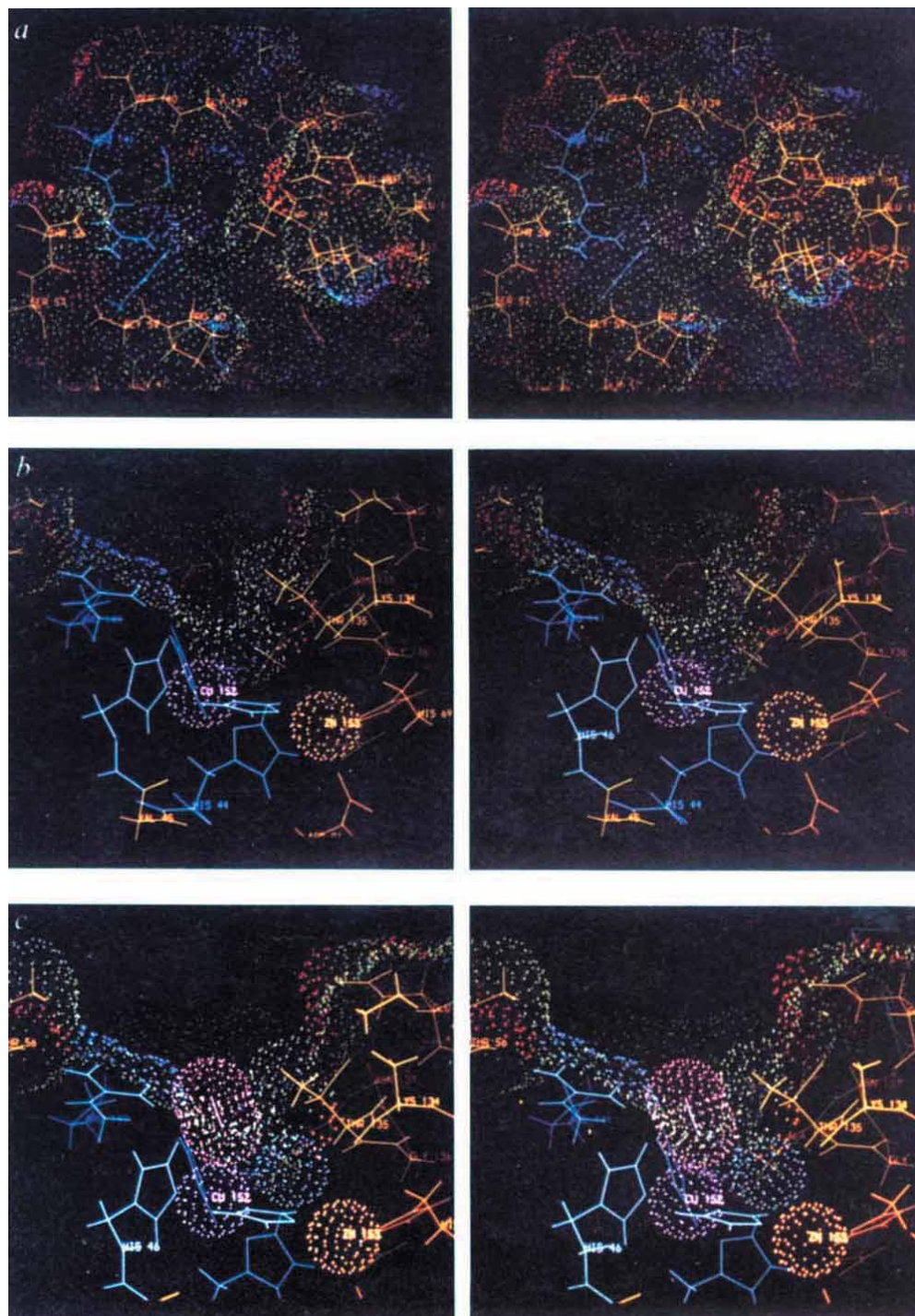
which were not apparent in earlier space-filling representations. One pit (the 'Cu site') is formed by the exposed surface of the Cu(II) and parts of His 61, His 118, Thr 135 and Arg 141. The adjacent pit (the 'water site') is formed by parts of the exposed surface of Thr 135, Gly 136, Ala 138, Gly 139 and Cu ligands His 44 and His 118.

Using interactive computer graphics, individually surfaced O_2^- (substrate) and CN^- (inhibitor) ions were manually docked into each of the two pits of the active-site channel molecular surface. O_2^- fitted the Cu site surface excellently (Fig. 2c), with one oxygen bound to the Cu (about 2 Å away) and the other H-bonded to Arg 141 NH1 (about 3 Å away). O_2^- fitted poorly into the water site, however, and made less chemical sense in terms of oxygen bonding geometry and Cu-O distance (~3.4 Å). CN^- could be fitted into either site with the required linear geometry.

Both active-site channel pits contain highly ordered water molecules (temperature factors <10 Å² and occupancies >0.9) in the refined structure. Two of those waters form a 'ghost' of an O_2^- positioned in the Cu site: one binds to the Cu (2.8 Å away) and the other to a guanidinium N of Arg 141 (3.3 Å

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Fig. 2 Stereo pairs showing the molecular surface and skeletal model (including hydrogens) of the active-site channel. Individual van der Waals radii were used (1.20 Å for H, 1.74 Å for C, 1.54 Å for N, 1.40 Å for O, 1.80 Å for S, and 1.4 Å for Cu and Zn). Atom coloured dots (where red = O, blue = N, green = C and H = colour of the atom to which it is bound) show the molecular surface accessible to a 1.4 Å probe sphere. The Cu and Zn were surfaced separately for clarity. O_2^- and CN^- were interactively docked into the enzyme active site using M. Connolly's display program, HANDLE. The O_2^- (O radius of 1.5 Å and O-O bond length of 1.27 Å) and CN^- (radii of 1.74 Å for C and 1.54 Å for N, and a C-N bond length of 1.15 Å) were surfaced independently. The terms front and back (or behind) refer to the stereo directions towards and away from the reader, respectively, while all other directional terms refer to locations within the plane of the page: *a*, Molecular surface of the active-site face of SOD. The Cu (magenta) and an adjoining pit (black) lie at the deepest part of the Y-shaped channel, surrounded by E 131, K 134, T 135, G 136, N 137, A 138 and G 139 at top and right, and by T 56, A 58, G 59, P 60, N 63 and R 141 at bottom and left. *b*, A thin slice (isolated by front and back clipping planes) through the active-site channel, viewed along the channel. Activity-important Arg 141 (ref. 30) is above and to the left of the Cu(II). Buried Asp 122 (yellow, lower right behind Zn) H-bonds to Cu ligand His 44 and Zn ligand His 69. Surface folds isolate specific pits above and next to the Cu. *c*, Superoxide (pink ellipsoid) docked into the enzyme active site, touching the Cu. The lower oxygen is surrounded by the surface of the Cu pit, while the upper oxygen H-bonds to Arg 141 (left) and touches Thr 135 (which could also H-bond if it rotated), but is open to solvent in front and back. A bound water (blue sphere) appears in the surface pit above and right of the Cu (the water site).



away). As expected for the geometry of a bound O_2^- , these waters form an O-O-Cu angle of 120° . The water pit holds a third water molecule placed to form a H-bond to the Cu-bound oxygen in the Cu pit. As this water is about 3.4 Å from the Cu, it does not appear to be an inner-sphere ligand.

The orientations of both side and main chains of the metal-liganding residues are stabilized by an interlocked network of H-bonds, some of which may have a functional relationship to the conformation and redox potential of the active site (Fig. 1). Of the 28 N and O atoms in the ligand residues, 8 are metal bound, 2 H-bond to solvent, and the other 18 all H-bond to other protein atoms. Extensive non-bonded contacts for the ligand residues were demonstrated by display of the atomic surfaces that packed closely enough together for a 0.25 Å radius probe to span the gap. The low temperature factors of the active channel residues, even where they are exposed at the surface,

reflect the order of this region. These tight interactions and the high degree of order suggest that, regardless of any possible strain implied by distortions from standard metal ligand geometry, large rearrangements of the ligands during catalysis are unlikely.

During SOD catalysis, the Cu is reversibly oxidized and reduced by successive encounters with O_2^- to yield O_2 and H_2O_2 by the following reaction equation¹⁴

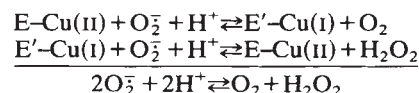


Figure 3 outlines a proposed model for the enzymatic mechanism that is based on SOD biochemistry, docking of an O_2^- in the active site and analysis of permitted movements of His 61. A

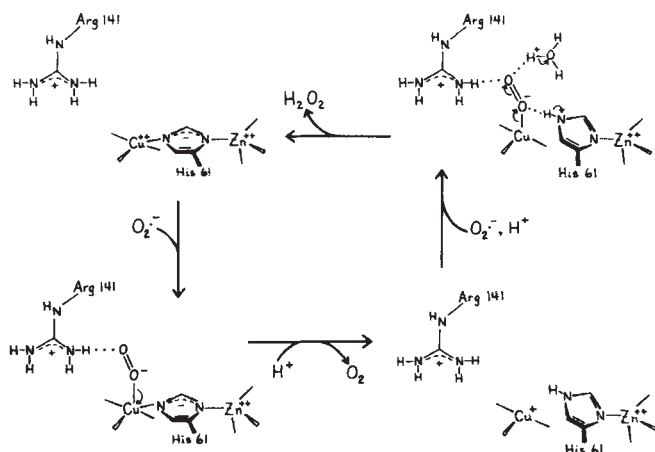


Fig. 3 Schematic mechanism. O_2^- displaces the axial H_2O binding one O to the Cu(II) and H-bonding the second O to a guanidinium N of Arg 141. As O_2^- is a stronger Cu ligand than H_2O , the mean ligand plane (see Fig. 4) can undergo a virtual shift, redefining the O_2^- as equatorial and His 46 as axial. Bound O_2^- reduces the Cu(II) to Cu(I) with simultaneous breaking of the bond between His 61 and the Cu; O_2 is released. [This first electron shift (from O_2^- to the Cu) is merely a resonance form of the initial O_2^- -Cu complex, and should be facilitated by the high Cu(II) redox potential.] His 61 NE2 becomes protonated, ND1 remains bound to the Zn, and the ring plane rotates out from the line joining the metals, making the Zn more tetrahedral. Additional slight shifts, probably including the buried Asp 122 carboxyl, can further improve tetrahedral geometry around both Cu(I) and Zn in a coordinated fashion (see also Fig. 1). All proposed movements are less than 1 Å. Binding of a second O_2^- to the Cu(I) then positions one O to H-bond with protonated His 61 NE2, while the other O again H-bonds to Arg 141. The Zn(II) bound to His 61 ND1 raises the pK_a of NE2 to ~13 (ref. 31), so unliganded NE2 will always be protonated at physiological pH. In our model building, the NE2 to superoxide O distance is 2.5 Å, consistent with a charged H-bond and partial proton transfer. Electron transfer from the Cu(I) coupled with proton transfer from His 61 forms Cu-hydroperoxide. Addition of a second proton from an active-site water gives the neutral H_2O_2 leaving group.

catalytic role is implied for the Zn, in addition to the previously proposed structural role¹⁵.

In oxidized SOD, the presence of the Zn and the tetrahedral distortion of the Cu(II) site increase the redox potential of the Cu(II) to 0.42 V (refs 16, 17), which is much higher than the 0.17 V for aqueous Cu(II) or the ~0.01 V for Cu(II)-histidine complexes¹⁸. In the first catalytic step, the transition is facilitated by relaxation (mediated through the His 61 bridge and the pair of H-bonds to Asp 122) of distortions in Zn-ligand geometry found for the Cu(II) enzyme. In the second step, the roles of the Zn are to ensure protonation of His 61 NE2 and to position this proton correctly for H-bonding and subsequent transfer to the second incoming O_2^- , thus completing the catalytic cycle. The Zn would enhance enzymatic activity without being essential, since His 61 NE2 will be at least partially protonated at pH 7 and protons are also available from water in the active channel.

Several features of this proposed mechanism are supported by other biophysical and biochemical studies. The narrow width of the channel (<4 Å) above the Cu is consistent with accessibility of the Cu to Cl^- (3.62 Å) and Br^- (3.90 Å), but not I^- (4.32 Å)¹⁹. The competitive anion inhibitors (CN^- , N_3^- and F^-) apparently bind by displacing a water molecule²⁰, as proposed for O_2^- binding from this work. X-ray absorption spectroscopy suggests that the Cu site becomes more tetrahedral on reduction²¹. The bond from bridging His 61 to the Cu apparently breaks during enzyme catalysis²², causing uncoupling of the metal sites²³ and the uptake of one proton per subunit⁶ by the His 61 ring N not liganded to the Zn(II)¹⁷. Moreover, while the

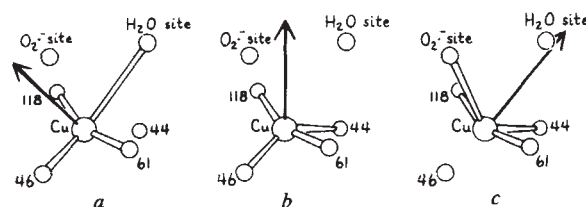


Fig. 4 Interconversion of axial and equatorial directions. The normal to the least-squares equatorial plane of the four Cu-liganding histidine N atoms is shown by the arrow in b, and the shift on anion binding to the water site (in a) or the Cu site (in c) is a redefinition of the equatorial plane to include the anion and exclude one of the other ligands. R.m.s. deviations of the equatorial ligand atoms from planarity are 0.32 Å in a, 0.42 Å in b and 0.34 Å in c, with a shift in the normal of 37° from b to a and 53° from b to c. Allowing for error, small realignments, and the differences between ESR parameters and planes calculated from coordinates, binding of CN^- in the water site is consistent with the single-crystal ESR data²⁶, giving a difference of 20° between the plane normal and g_z for the CN^- derivative compared with a difference of 9° for the native enzyme.

catalytic rate of native SOD is independent of pH over the range 5–9.5 (ref. 14) (indicating that the protons required by the reaction stoichiometry do not come from bulk water), the activity of the Zn-free derivative is pH-dependent, with an apparent pK_a at 6.9 (ref. 24), which may reflect the pK_a of His 61.

In the refined crystallographic model of the active site, the geometry of the Cu coordination indicates that replacement of the H_2O molecule in either binding site by a stronger ligand would allow a redefinition of the equatorial plane to include that solvent-accessible binding position. As either new plane would be less distorted (Fig. 4), such a virtual rearrangement would result in more symmetrical Cu site geometry as observed in the more axial ESR spectra with anion inhibitors²⁵. The axial-equatorial interconversion could be very rapid, and explains these ESR data without requiring the large physical rearrangements of ligands previously proposed^{26,27}.

The model was fitted to the electron density map on the GRIP-75 molecular graphics system built by the University of North Carolina Department of Computer Science. This stage of refinement was initiated at the National Bureau of Standards with the assistance of A. Wlodawer and completed using R. Fletterick's VAX computer at the University of California at San Francisco. Molecular surfaces were calculated with the assistance of M. Connolly and displayed at the University of California at San Francisco computer graphics facility directed by R. Langridge and managed by T. Ferrin. This work was supported by NIH.

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1. Fridovich, I. *Adv. inorg. Biochem.* **1**, 67–90 (1979).
2. Tainer, J. A., Getzoff, E. D., Beem, K. M., Richardson, J. S. & Richardson, D. C. *J. molec. Biol.* **160**, 181–217 (1982).
3. Richardson, J. S. *Nature* **268**, 495–500 (1977).
4. Hermans, J. & McQueen, J. E. *Acta crystallogr.* **A30**, 730–739 (1974).
5. Wlodawer, A. & Hendrickson, W. A. *Acta crystallogr.* **A38**, 239–247 (1982).
6. Getzoff, E. D. thesis, Duke Univ. (1982).
7. Connolly, M. L. *Science* **221**, 709–713 (1983).
8. Connolly, M. L. *Quantum Chemistry Program Exchange Bull.* **1**, 75 (1981).
9. Richards, F. M. *A. Rev. Biophys. Bioeng.* **6**, 151–176 (1977).
10. Steinman, H. M., Naik, V. R., Abernethy, J. L. & Hill, R. L. *J. biol. Chem.* **249**, 7326–7338 (1974).
11. Steinman, H. M. *J. biol. Chem.* **255**, 6758–6765 (1980).
12. Jabusch, J. R., Farb, D. L., Kerschensneider, D. A. & Deutsch, H. F. *Biochemistry* **19**, 2310–2316 (1980).
13. Lerch, K. & Ammer, D. *J. biol. Chem.* **256**, 11545–11551 (1981).
14. Klug, D., Rabani, J. & Fridovich, I. *J. biol. Chem.* **247**, 4839–4842 (1972).
15. Valentine, J. S. & Pantoliano, M. W. in *Metal Ions in Biology* Vol. 3 (ed. Spiro, T. G.), 291–358 (Wiley, New York, 1981).
16. Lawrence, G. D. & Sawyer, D. T. *Biochemistry* **18**, 3045–3050 (1979).
17. Morpurgo, L., Giovagnoli, C. & Rotilio, G. *Biochim. biophys. Acta* **322**, 204–210 (1973).
18. Margerum, D. W., Cayley, G. R., Weatherburn, D. C. & Pagenkopf, G. K. in *Coordination Chemistry* Vol. 12 (ed. Martell, A. E.) 1–220 (American Chemical Society, Washington DC, 1978).
19. Rigo, A., Stevanato, R., Viglino, P. & Rotilio, G. *Biochem. biophys. Res. Commun.* **79**, 776–783 (1977).

20. Boden, N., Holmes, M. C. & Knowles, P. F. *Biochem. J.* **177**, 303–309 (1979).
 21. Blumberg, W. E., Peisach, J., Eisenberger, P. & Fee, J. A. *Biochemistry* **17**, 1842–1846 (1978).
 22. McAdam, M. E. *et al. Biochem. J.* **167**, 271–274 (1977).
 23. Rotilio, G., Calabrese, L., Mondovi, B. & Blumberg, W. E. *J. biol. Chem.* **249**, 3157–3160 (1974).
 24. Burger, A. R. thesis, Columbia Univ. (1979).

25. Beem, K. M., Richardson, D. C. & Rajagopalan, K. V. *Biochemistry* **16**, 1930–1936 (1977).
 26. Lieberman, R. A., Sands, R. H. & Fee, J. A. *J. biol. Chem.* **257**, 336–344 (1982).
 27. Van Camp, H. L., Sands, R. H. & Fee, J. A. *Biochim. biophys. Acta* **704**, 75–89 (1982).
 28. Johnson, C. K. *Oak Ridge natn. Rep.* ORNL-3794 (1965).
 29. Valentine, J. S., Sheridan, R. P., Allen, L. C. & Kahn, P. C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1009–1013 (1979).
 30. Malinowski, D. P. & Fridovich, I. *Biochemistry* **18**, 5909–5917 (1979).
 31. Martin, R. B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4346–4347 (1974).

Electrostatic recognition between superoxide and copper, zinc superoxide dismutase

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Electrostatic forces have been implicated in a variety of biologically important molecular interactions including drug orientation by DNA¹, protein folding^{2–3} and assembly⁴, substrate binding and catalysis^{5–7} and macromolecular complementarity with inhibitors, drugs and hormones^{8–11}. To examine enzyme–substrate interactions in copper, zinc superoxide dismutase (SOD), we developed a method for the visualization and analysis of an enzyme's three-dimensional electrostatic vector field that allows the contributions of specific residues to be identified. We report here that the arrangement of electrostatic charges in SOD promotes productive enzyme–substrate interaction through substrate guidance and charge complementarity: sequence-conserved residues create an extensive electrostatic field that directs the negatively charged superoxide (O_2^-) substrate to the highly positive catalytic binding site at the bottom of the active-site channel. Dissection of the electrostatic potential gradient indicated the relative contributions of individual charged residues: Lys 134 and Glu 131 seem to have important roles in directing the long-range approach of O_2^- , while Arg 141 has local orienting effects. The reported methods of analysis may have general application for the elucidation of intermolecular recognition processes.

At physiological pH, SOD has a net negative charge that varies with species: *pI* values range over 4.6–6.8 (ref. 12). The

electrostatic barrier to incoming O_2^- resulting from the enzyme's net negative charge should diminish with increasing ionic strength, thus accelerating the reaction. Instead, raising the ionic strength slows catalysis in the native enzyme, implying that local electrostatic forces facilitate the reaction¹³. Reversal of this ionic strength dependence after acylation of seven or eight lysine residues per subunit (15% residual activity) suggests that local positive charges from one or more lysines are important in providing long-range electrostatic guidance for O_2^- . Charge guidance was also suggested from studies of the enzyme's dipole and overall electrostatic potential (as calculated *in vacuo* from charged atoms only)¹⁴. Neutralization of active-site Arg 141 with phenylglyoxal causes a 90% reduction in activity¹⁵, but does not affect the ionic strength response¹³; these results are compatible with short-range electrostatic stabilization of O_2^- by Arg 141 at the binding site, as proposed from computer graphics modelling (see accompanying paper¹⁶).

The electrostatic contributions to enzyme–substrate recognition in SOD were analysed by calculation and colour computer graphics display of the electrostatic potential and the electrostatic field vectors in the active-site channel. The molecular surface of SOD colour-coded by electrostatic potential (Fig. 1) shows no organized pattern over most of the surface, but reveals a striking positive region which extends over the long, deep channel above the catalytic Cu ion. As would be expected, the positive potential near the Cu ion is very high, forming a complementary binding site for the negatively charged O_2^- substrate. The extent of the pattern of positive potential ($550 \text{ Å}^2 > +7 \text{ kcal mol}^{-1}$) suggests that the sequence-conserved amino acids of the active-site channel¹⁶ also have an electrostatic role in attracting O_2^- .

The rate constant for SOD dismutation of O_2^- ($2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$)¹⁷ is about 10% of the maximum diffusion-controlled limit calculated from collision theory¹⁸, neglecting steric and orientation factors. This is remarkable, as the catalytic Cu ion forms only about 0.1% of the enzyme's molecular surface. However, the entire active-site channel forms about 10% of the surface, so the observed rate constant could be rationalized by assuming that all collisions with the active-site channel are productive. More probably, electrostatic forces provide some pre-collision orientation and/or the enhancement of diffusion.

Table 1 Effects of individual residues on the electrostatic field direction

Residue(s) neutralized*	Average angular change (deg) in electrostatic field direction produced in each of the eight shells								
	0 Å	2 Å	4 Å	6 Å	8 Å	10 Å	12 Å	14 Å	All
Zn, His 61	1.3	4.1	1.9	2.1	3.1	3.9	4.3	5.8	4.4
Glu 119	0.6	1.7	3.1	5.5	7.1	8.8	8.6	8.7	8.1
Lys 120	0.7	2.0	5.3	7.5	18.0	22.6	26.3	24.5	22.4
Glu 130	0.2	0.5	1.0	2.0	3.9	6.1	9.7	19.0	10.7
Glu 131	0.6	3.0	10.1	23.1	30.7	37.4	37.9	36.9	34.7
Lys 134	0.4	1.2	2.7	5.6	11.9	20.3	31.0	37.3	26.7
Arg 141	7.4	16.0	31.9	29.3	21.7	23.9	24.5	20.7	23.2
Glu 119 and Lys 120	0.4	1.4	4.2	4.8	13.4	16.8	19.6	17.1	16.3
Glu 131 and Lys 134	0.3	2.1	7.9	17.5	25.0	34.2	43.4	44.2	37.3
<i>D</i> = 80	4.8	6.4	13.6	9.7	12.8	14.3	16.7	18.2	15.8

The shell is named by the radial distance from the position of the axial water bound to the Cu(II), with the number of vectors calculated in each successive shell being: 1, 5, 10, 14, 32, 65, 105 and 107. *D* = 80, changes resulting from the use of the bulk water constant dielectric of 80.

* Mathematically neutralized for calculating the electrostatic field.

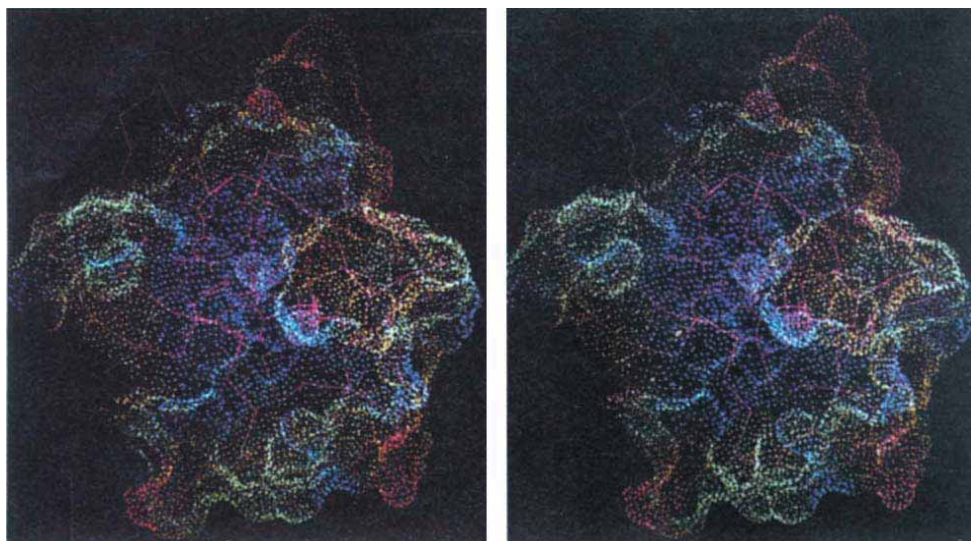
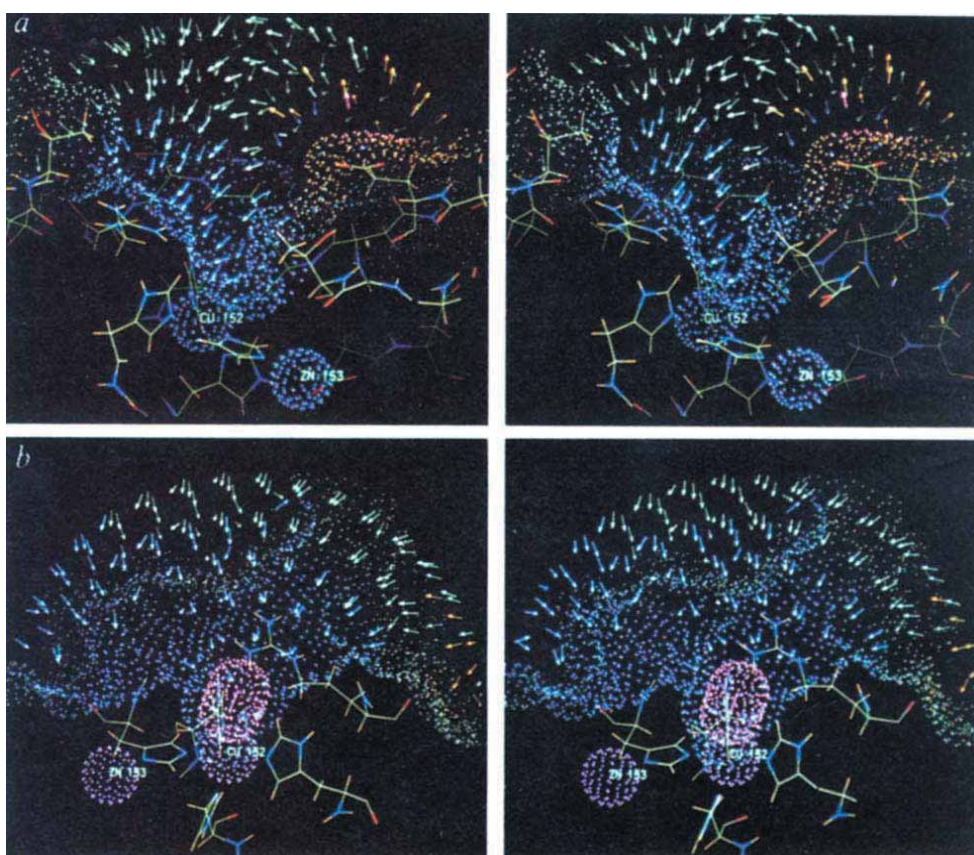
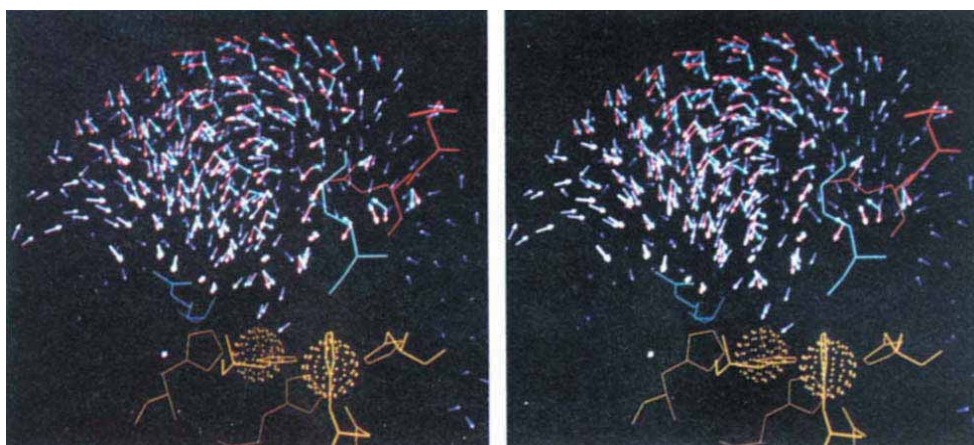
**Fig. 1****Fig. 2****Fig. 3**

Fig. 1 The electrostatically colour-coded active-site face of the SOD subunit. The molecular surface²² is shown as dots coloured by electrostatic potential (red, < -21 kcal mol⁻¹; yellow, -21 to -7 kcal mol⁻¹; green, -7 to $+7$ kcal mol⁻¹; cyan, $+7$ to $+21$ kcal mol⁻¹; blue > 21 kcal mol⁻¹). The skeletal model Cu ligands and active-site Arg 141 (centre) can be seen together with the Cu (purple sphere) in the most positive part of the active channel. The side chain of Lys 134 (just below and right of the Cu) projects forward. Examination of the entire surface shows that this large positive region is unique to the active-site channel; most residues in this area are invariant in the known SOD sequences¹⁶. The second active site in the dimer (related by an approximately vertical 2-fold axis) is oriented to allow productive collisions from the opposing direction. Partial charges were assigned to the atomic positions of the refined developed 2 Å crystal structure of the bovine erythrocyte enzyme¹⁶ using the AMBER data base^{8,23}. This dictionary of Mulliken net atomic charges was developed from *ab initio* LCAO-SCF-MO (linear combination of atomic orbitals-self-consistent field-molecular orbital) calculations on individual amino acids using the STO-3G basis set (three gaussian functions per Slater-type orbital)⁵. All non-hydrogen atoms and all potentially H-bonding hydrogens were included explicitly, while a united-atom representation was used for aliphatic and aromatic hydrogens. The amino acid residues were assumed to be in their predominant state of ionization in aqueous solution at physiological pH. Liganding histidines were protonated at their non-liganding N, His 41 at both ring N (which are both H-bond proton donors) and His 19 at NE2. Due to the acetyl blocking group, the N terminus was left uncharged. The Cu and Zn were each assigned a charge of +2, as in the oxidized enzyme. The resultant net charge on the dimeric enzyme was -4. No solvent water molecules were included in the calculation. The electrostatic potential V , resulting from the partial charges q_i assigned to the atomic positions r_i , was determined at each surface point r_0 by the following equation: $V = \sum_i q_i / D |r_i - r_0|$, where D is the dielectric constant for a constant dielectric model, or is replaced with $D|r_i - r_0|$ for a dielectric model with linear distance dependence⁹. Using the latter model, the electrostatic potential assigned to each molecular surface point was calculated 1.4 Å above that point along a surface normal vector; this calculation point (r_0) represents the centre of a solvent probe sphere at its closest approach to the protein. The contributions of each partial charge within 20 Å of r_0 were summed. To correct for dipoles split by the finite cut-off radius, we used the method of neutral spheres^{23,24}: a compensatory charge placed at the cut-off radius ensures zero net charge inside the sphere.

Fig. 2 The deep active-site channel of SOD with electrostatically colour-coded molecular surface and electrostatic field vectors showing the guidance of O₂⁻ (pink ellipsoid) into the catalytic site: *a*, Channel in cross-section and *b*, as a slice along the channel. The vectors were calculated at points outside the molecular boundary, on concentric spheres of up to 14 Å radius centred on the axial water ligand of the Cu ion. Both the molecular surface (dots) and the field vectors are colour-coded by electrostatic potential as in Fig. 1. The skeletal model includes hydrogens and is coloured by atom type (where red = O, blue = N, green = C and yellow = H). As shown in *b*, the electrostatic potential remains positive farthest from the Cu ion in a pathway passing above the Zn from the far left to the Cu. The electrostatic field, or electrostatic potential gradient (the magnitude and direction of maximum change in the potential), was calculated by evaluating the partial derivative of the potential with respect to each of the three coordinate axes. For the x component with a distance-dependent dielectric model: $dV/dx = \sum_i -2q_i|x_i - x_0|/D|r_i - r_0|^4$. By convention, electrostatic field vectors indicate the direction in which a positive charge would move in the field; we reversed this convention here to show the directional force on the negatively charged O₂⁻.

Fig. 3 The contribution of Lys 134 to the electrostatic field in the active-site channel. Lys 134 (right) and Arg 141 (left) are shown in blue, Glu 130 and 131 (far right) in red, and the Cu and Zn and their ligands in yellow (bottom). Two sets of electrostatic field vectors indicate the direction in which an O₂⁻ would be guided in native SOD (blue arrows) and in SOD with the charge on sequence-conserved Lys 134 neutralized (red arrows). Where the electrostatic field direction is not affected by the neutralization of Lys 134, vector pairs are superimposed (magenta arrows). This stereo pair was generated at the Research Institute of Scripps Clinic using the graphics language GRAMPS of O'Donnell and Olson²⁵.

To evaluate long-range guidance effects on approaching O₂⁻ molecules, we calculated and displayed electrostatic field vectors within and beyond the SOD active-site channel (Fig. 2). Each arrow was colour-coded by the electrostatic potential at its origin, as coding by field magnitude showed primarily that the field decreased with distance from the molecular surface. The electrostatic field vectors indicate that attraction of O₂⁻ is a long-range process occurring even beyond the active-site channel. The direction of vector flow indicates that an O₂⁻ would be repelled from the banks of the channel into the channel itself, where it would be swept along before diving down to dock next to the positive charges of the Cu²⁺ and activity-important Arg 141.

Colour-coding of the field vectors by electrostatic potential showed the direction in which the potential remains positive farthest from the Cu²⁺ (Fig. 2*b*). An O₂⁻ travelling in this direction passes near the positively charged ϵ -amino group of sequence-conserved Lys 134 located 13 Å from the Cu at one end of the active-site channel (see Fig. 1). Non-productive association of O₂⁻ with Lys 134 is reduced by the adjacent negatively charged carboxylate of sequence-conserved Glu 131. The strategic placement of this pair of opposite charges helps direct the field vectors into the active channel. Within the channel, O₂⁻ is attracted towards the positive charges on Cu²⁺, Zn²⁺ and Arg 141; the potential is most positive on the axial water position above the Cu ion. The electrostatic orientation imposed by these residues should increase the number of O₂⁻ collisions with SOD that lead to catalysis.

Although the calculated magnitude of the electrostatic potential associated with each arrow depends on the dielectric model used, potentials along the pathway described above remain greater than the average kinetic energy ($3/2 kT = 0.9$ kcal mol⁻¹) even when damped by the use of the dielectric constant of bulk water ($D = 80$). Thus, the equilibrium concentration of O₂⁻ near the active-site channel will be significantly greater than that near other regions of the protein, where the potential is more often negative.

Charged residues near the active-site channel were selectively examined to evaluate their contributions to electrostatic substrate guidance. The residues were individually neutralized by appropriately reassigning their component partial charges⁶; the field resulting from each modified set of charges was compared with the originally calculated field (Table 1). The largest angular changes in the electrostatic field vector directions are seen for Arg 141, Lys 134, Glu 131 and Lys 120. Neutralization of Glu 119, Glu 130 or the Zn²⁺ (concurrently with neutralization of bridging His 61) produces smaller, more localized changes. Arg 141 mainly affects the field direction near the Cu ion; this is consistent with its local role in binding O₂⁻. Figure 3 illustrates the large shifts in the field vector directions that occurred throughout much of the active channel on neutralization of sequence-conserved Lys 134. Both this residue and sequence-conserved Glu 131 appear to be major contributors to the long-range electrostatic guidance of O₂⁻ to the catalytic site, as described above. Although sequence-variable Lys 120 has a significant individual effect, it always occurs in conjunction with Glu 119 and their joint effect is small.

Due to uncertainty in the correct choice of a dielectric model^{1,7,19-21}, we examined the potential gradient near the SOD active site with different linearly distance-dependent ($D = r, 2r, 4r$) and constant ($D = 1, 5, 10, 20, 80$) dielectric models. The choice of dielectric model had a much greater effect on the calculated magnitude of both the electrostatic potential and the electrostatic field, than on the calculated direction of the field. Conversion between distance-dependent and constant dielectric models causes an average change of only 15.8° in the field vector directions in the SOD active channel (see last line of Table 1), while changes in the magnitude of the dielectric constant leave the vector directions unchanged. Thus, despite problems in quantitating the electrostatic effects, the mechanism and pathway for substrate guidance implied by the SOD electrostatic field stay largely unchanged regardless of the dielectric model.

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1. Dean, P. M. *Br. J. Pharmac.* **74**, 39–46 (1981).
2. Hol, W. G. J., Halie, L. M. & Sander, C. *Nature* **294**, 532–536 (1981).
3. Sheridan, R. P., Levy, R. M. & Salemm, F. R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4545–4549 (1982).
4. Perutz, M. F. *Science* **201**, 1187–1191 (1978).
5. Hayes, D. M. & Kollman, P. A. *J. Am. chem. Soc.* **98**, 7811–7816 (1976).
6. Sheridan, R. P. & Allen, L. C. *J. Am. chem. Soc.* **103**, 1544–1550 (1981).
7. Warshel, A. & Levitt, M. *J. molec. Biol.* **103**, 227–249 (1976).
8. Blaney, J. M. *et al. J. Am. chem. Soc.* **104**, 6424–6434 (1982).
9. Weiner, P. K., Langridge, R., Blaney, J. M., Schaefer, R. & Kollman, P. A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3754–3758 (1982).
10. Pullman, B., Lavery, R. & Pullman, A. *Eur. J. Biochem.* **124**, 229–238 (1982).
11. Friend, S. H., March, K. L., Hanania, G. I. H. & Gurd, F. R. N. *Biochemistry* **19**, 3039–3047 (1980).
12. Salin, M. L. & Wilson, W. W. *Molec. cell. Biochem.* **36**, 157–161 (1981).
13. Cudd, A. & Fridovich, I. *J. biol. Chem.* **257**, 11443–11447 (1982).
14. Koppenol, W. H. in *Oxygen and Oxy-Radicals in Chemistry and Biology* (eds Powers, E. L. & Rodgers, M. A. J.) 671–676 (Academic, New York, 1981).
15. Malinowski, D. P. & Fridovich, I. *Biochemistry* **18**, 5905–5917 (1979).
16. Tainer, J. A., Getzoff, E. D., Richardson, J. S. & Richardson, D. C. *Nature* (this issue).
17. Klug, D., Rabani, J. & Fridovich, I. *J. biol. Chem.* **247**, 4839–4842 (1972).
18. Margerum, D. W., Cayley, G. R., Weatherburn, D. C. & Pagenkopf, G. K. in *Coordination Chemistry Vol. 2* (ed. Martell, A. E.) 1–220 (American Chemical Society, Washington DC, 1978).
19. Hopfinger, A. J. in *Conformational Properties of Macromolecules* (eds Horecker, B., Kaplan, N. O., Marmur, J. & Scheraga, H. A.) 39 (Academic, New York, 1973).
20. Gelin, B. R. & Karplus, M. *Biochemistry* **18**, 1256–1268 (1979).
21. Dearing, A., Weiner, P. & Kollman, P. A. *Nucleic Acids Res.* **9**, 1483–1497 (1981).
22. Connolly, M. L. *Science* **221**, 709–713 (1983).
23. Weiner, P. K. & Kollman, P. A. *J. comp. Chem.* **2**, 287–303 (1981).
24. Adams, D. J. *Chem. phys. Lett.* **62**, 329–333 (1979).
25. O'Donnell, T. J. & Olson, A. J. *Computer Graphics* **15**, 133–142 (1981).

Expression of *Klebsiella* nitrogen fixation genes in *Azotobacter*—a caution

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IN 1976 we reported in this journal¹ the correction of structural *nif* mutations of *Azotobacter vinelandii* (strains UW1, 10, 100 and 38) by a plasmid RP41 (now called pRD1) which carries the *nif* cluster of *Klebsiella pneumoniae*.

We are now obliged to report that we cannot repeat this effect. The original Nif⁺ transconjugant *Azotobacter* strains were lost and in several attempts to obtain new ones we have used both pRD1 and a derivative, pMF250, which is sometimes more stable in intergeneric transfers. Transconjugants have readily been obtained which retain *nif* according to transfer of the plasmid back to *Escherichia coli* but none shows a Nif⁺

phenotype either in growth or acetylene reduction tests, performed in a variety of test conditions.

We have re-examined our paper and our experimental records and are at a loss to explain this discrepancy. Our original controls, which included reversion to the Nif⁺ phenotype on eliminating the plasmid and also immunospecific recognition of the peptides of the *Klebsiella* molybdoprotein in the corrected *azotobacters*, seem to eliminate trivial explanations, such as reversion of the *nif* mutants or inadequate separation of donor and recipient organisms. A substantial number of successful transconjugations were performed and human error is therefore unlikely. We are compelled to conclude either that some systematic error, which still eludes us, occurred in our early experiments or that a special nutritional or other condition, favouring *nif* expression, existed fortuitously which we have not been able to reproduce.

We must therefore inform your readers that our evidence for correction of structural *nif* mutations in *A. vinelandii* by *Klebsiella nif* is subject to reasonable doubt. We much regret that our publication will have misled others as much as ourselves. The recent evidence for function of *Klebsiella nifA* in *A. vinelandii*² is, of course, unaffected by these considerations and remains valid.

1. Cannon, F. & Postgate, J. *Nature* **260**, 271–272 (1976).
2. Kennedy, C. & Robson, R. L. *Nature* **301**, 626–628 (1983).

IQ in Japan and the United States

LYNN¹ recently reported that the average intelligence quotient (IQ) in Japan exceeds that in the United States and suggested that the disparity has been growing during this century. Several earlier studies by Lynn had yielded similar, but less extreme, results^{2,3}. Because of the potential importance of these reports it is useful to examine the methodology upon which they are based. We suggest that Lynn's conclusions are based on a flawed research procedure.

Lynn sought to compare national averages in intelligence by evaluating performance in Japan according to American norms, and asked what scores children would receive if their responses were scored according to the American norms. Specifically, he compared data obtained from the groups on which the test was standardized in Japan with data obtained from the American standardization groups. Such a procedure may be meaningful if the standardization groups are equally representative of the populations of each country, but in this case they are not.

An intelligence test must be standardized to obtain norms against which scores on the test can be interpreted. That is, the test must be given to a representative sample of individuals from the population in which the test is to be used. Norms are constructed on the basis of the responses of this standardization group. We have examined the manuals describing the standardization of the Japanese and American versions of the 1974 revision of the Wechsler Intelligence Scale for Children (WISC-R), the test used by Lynn in his most recent analyses.

The American sample of 2,200 children, 100 boys and 100 girls at each age level from 6 to 16 yr, was carefully selected according to six variables: age, sex, race, geographical region, occupation of head of household and urban-rural residence⁴. Kodama *et al.*⁵, who standardized the test in Japan, selected their standardization sample according to only three of these variables: geographical region, age and sex. Japan was divided into three regions, north, middle and south, and samples proportional to the population of the region were selected from each region to yield a final sample of 50 boys and 50 girls at each age level. Occupation of head of household and urban-rural residence were completely ignored. The rationale for these omissions was that these variables were "difficult to consider" or "unnecessary in Japan"⁵.

Lynn is not correct, therefore, when he writes that the standardization procedure for Japan "followed that in the United

States in matching the sample for socio-economic status to the total population"². This is an important omission. It has long been known from Western studies that correlations between school-age children's IQs and parents' educational and occupational levels tend to be around 0.50 (ref. 6). This conclusion also applies to Japan. In a recent study⁷, socio-economic status of the family was found to be a significant predictor of children's performance on intelligence tests. At age 6, for example, the correlation between SES and Binet IQ was 0.56; at age 11, the correlation between SES and WISC-R IQ was 0.46.

Urban-rural differences in performance, as they are related to occupational and educational levels, are typically found in studies of intelligence tests. Several Japanese studies have revealed such differences. A study conducted in the early 1970s reveals that the average *T*-score point of rural children's IQs was 45.9, which is around 7 IQ points lower than the national norm (ref. 8, p. 352). Sugimura also has recently reported significant differences between the intelligence test scores of Japanese rural and urban children⁹.

We do not present these data to account for part or all of the disparity of 11 IQ points between Japanese and American children claimed by Lynn, but rather to show that these variables should have been taken into consideration in standardizing the test in Japan. Because they were not, comparison of numerical scores from the two standardization samples have little meaning.

Educational and occupational status differ in Japan according to urban-rural residence in a manner similar to that found in other countries. For example, whereas 56% of the personnel in all industries in Japan had completed secondary and/or higher education, only 23% in agriculture, forestry and fishery—primarily rural occupations—had done so⁵. While Japanese society has become increasingly urban, a reasonably large percentage still resides in the countryside. According to the 1975 census of Japan, 75.9% of the population lived in *shi* (cities), while 24.1% of the population resided in *mura* or *machi* (villages and small towns)¹⁰. Indeed, it has been estimated by prefectural authorities that as late as 1978, 22.6% of elementary schools in Japan were in remote isolated areas¹¹.

The exact composition of the Japanese sample used in standardizing the WISC-R cannot be ascertained from the description provided by Kodama *et al.*, but in reviewing the names of the schools with which the cooperating teachers were associated, a clear bias towards an urban sample is apparent. The sample was obtained by seeking the cooperation of teachers

primarily from large cities and their suburbs and from prefectural capitals. Of 49 cooperating teachers, 45 (92%) were employed in cities with a population greater than 50,000. In Japan, 64% of the population resides in cities of such size. No teachers were from *mura* or *machi*, even though 24% of the Japanese population lives in these villages and small towns.

The decision by Kodama and his colleagues to concentrate on urban populations was appropriate for constituting a normative group that characterized the situations in which the WISC-R would most likely be used in Japan. However, the standardization procedure was not one that permits generalizations of the type Lynn has made about the intellectual characteristics of the population of 6–16 year olds in Japan.

The sampling method used in Japan has apparently resulted in a sample biased for higher socio-economic status and urban residence compared with the national population of Japan. Supporting the proposal that a broader range of the population was sampled in the United States is the fact that the standard deviation of the performance IQ was larger in the United States than in Japan (10.89 versus 9.30). Values for the standard deviation of the total IQ were also larger in the United States: 21.01 versus 19.19 (Table 6 in ref. 4 and Table 3 in ref. 5).

Other factors also limit generalizations that can be made about Japanese and American IQs from the WISC-R. Although the WISC-R contains verbal and performance subtests, only the performance and Digit Span subtests were used by Lynn. Cross-national comparison of IQs on the basis of the performance IQ alone is not appropriate. The performance score has a different meaning and interpretation from that of the Full Scale IQ. This is especially the case when the correlation between the two scores is lower (0.81) in Japan than in the United States (0.90).

The reliability and internal consistency of the test also differ between the two countries. The overall split-half reliability of the performance scale is 0.90 in the United States and 0.85 in Japan (Table 9 in ref. 4 and Table 6 in ref. 5). Even more pronounced is the difference in the correlation between the performance and verbal subtests. The correlation is markedly lower in Japan than in the United States, 0.44 versus 0.67. (Attenuation does not characterize all intercorrelations in Japan, however; the average intercorrelation of the performance subtests is very similar in the two countries, 0.57 in Japan and 0.53 in the United States.) In view of these differences, one can be less confident about describing intellectual functioning on the basis of the WISC-R performance test in Japan than in the United States. These differences in the statistical proper-

ties of the test in the two countries are interesting, but will require further research for their explication.

The title of Lynn's paper which suggests the existence of a 'great disparity' in IQ is seriously misleading. The average IQ of any population is 100 (1.00×100). The intelligence quotient is defined as the level of mental functioning expressed in terms of age divided by the child's chronological age. Intelligence tests are purposefully constructed so that the value of this quotient is unity for each chronological age within a given population from which the standardization sample was recruited.

We conclude that there are serious flaws in Lynn's attempt to compare the IQs of American and Japanese children. Several critical factors were overlooked, the most critical being the lack of representativeness of the standardization sample in Japan. We argue, therefore, that the lack of comparability of the standardization samples precludes the type of comparison Lynn attempted to undertake. The relative status of American and Japanese intellectual functioning remains unknown.

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1. Lynn, R. *Nature* **297**, 222 (1982).
2. Lynn, R. *Bull. Br. psychol. Soc.* **30**, 69 (1977).
3. Lynn, R. & Dziobon, J. J. *Person. Indiv. Diff.* **1**, 95 (1980).
4. Wechsler, D. *Wechsler Intelligence Scale for Children—Revised* (Psychological Corporation, 1974).
5. Kodama, H., Shinagawa, F. & Motegi, M. *Wechsler Intelligence Scale for Children—Revised* (Nihon Bunka Kagakusha, Tokyo, 1978).
6. Bayley, N. in *Carmichael's Manual of Child Psychology* (ed. Mussen P.) 1163–1210 (Wiley, New York, 1970).
7. Azuma, H., Kashiwagi, K. & Hess, R. D. *Children's Cognitive Development and Mothers' Attitudes and Behavioral Styles* (in Japanese) (Tokyo Daigaku Shuppankai, 1981).
8. Mizoguchi, K. *Annual Review of Japanese Child Psychology* (in Japanese) (Kaneko Shobo, 1979).
9. Sugimura, T. *Jap. J. Educ. Psychol.* **28**, 324 (in Japanese) (1980).
10. *Yearbook, Statistics Department, Office of the Prime Minister* (in Japanese) (1979).
11. *Education in Japan* (Ministry of Education, Science and Culture, 1979).

LYNN REPLIES—Both Stevenson and Azuma and Flynn¹ have suggested my calculation of the mean Japanese at 111 is too high and requires revision downwards. The principal point made by Stevenson and Azuma is that the Japanese sample for the standardization of the Wechsler Intelligence Scale for Children (WISC-R) was drawn from urban schools only, that the mean intelligence quotient

(IQ) in rural schools is lower and that consequently the mean IQ of Japanese children derived from the WISC-R was biased upwards. They suggest a disparity of 7 IQ points between Japanese urban and rural children on the basis of a study by Sato and Hario, but no details of the adequacy of this study are given. They do not cite the study by Hattori *et al.*² which showed no significant differences in mean IQ between rural and urban Japanese children. Thus it is open to doubt whether the omission of rural children in the Japanese standardization of the WISC-R resulted in any serious distortion of the mean IQ in Japan. If, however, the figures suggested by Stevenson and Azuma are accepted it is quite feasible to recalculate the mean Japanese IQ making allowance for the alleged bias in the standardization. Such a recalculation is given at the end of this communication.

The second point made by Stevenson and Azuma concerns the use of the performance scale and omission of the verbal scale in the calculation of the mean Japanese IQ. It is difficult to see that the slightly lower correlation between the two scales in Japan (0.81 compared with 0.90 in the USA) and other small statistical differences between the two samples can seriously affect the comparison. In any case the inclusion of the arithmetic and digit span subtests in the calculation of the mean Japanese IQ by Flynn goes some way to correcting the omission and means that the Japanese IQ can now be calculated from all the major specific abilities except verbal. Japanese verbal ability remains an unknown quantity but a figure based on the other major specifics must give a close approximation to the true value of general intelligence.

Turning now to the arguments advanced by Flynn, the first point is that the mean American IQ should be raised by 2.26 points because of the low means obtained by racial minorities in the US. Hence a comparison of the Japanese with white Americans only reduces the Japanese advantage by 2.26 IQ points. This is indisputably correct and I made the same point myself in an earlier paper on Japanese intelligence³. Second, inclusion of the arithmetic and digit span tests reduces the mean Japanese IQ by approximately 1 IQ point. This is also correct. Third, the American standardization was carried out in 1972 and the Japanese in 1975. It is argued that the American mean IQ has been increasing at approximately 0.32 points per year, and therefore that on this account an additional 1 point must be

taken off the Japanese mean. While this point may be more contentious, taking the three arguments together the Japanese mean IQ should be reduced from 110.7 to 106.6.

If the value of 106.6 is accepted, a further correction to adjust for the urban sampling bias suggested by Stevenson and Azuma can be made as follows. Taking their figures of a mean IQ for rural children of 7 points below the national norm and the proportion of rural children in Japan at 24%, the mean IQ for all Japanese children will be

$$\frac{97.39 \times 24 + 106.6 \times 76}{100} = 104.39$$

It is therefore suggested that taking into account all the arguable qualifications proposed by Stevenson and Azuma and by Flynn, the mean IQ of the present generation of Japanese young people is approximately 104.4. This value remains significantly higher than the mean of 100 for American Caucasians.

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1. Flynn, J. R. *Nature* **301**, 655 (1983).
2. Hattori, K., Misawa, G. & Fujita, K. *Natn. Rehab. Res. Bull. Jap.* **3**, 149 (1982).
3. Lynn, R. *Bull. Br. psychol. Soc.* **30**, 69 (1977).

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

Biology meets politics

Leon Eisenberg

How Humans Adapt: A Biocultural Odyssey.

Edited by Donald J. Ortner.

Smithsonian Institution Press: 1983. Pp. 560. Hbk \$19.95; pbk \$9.95.

PUBLISHED symposia devoted to grand and over-arching themes offer readers an opportunity to confirm for themselves the proposition that the whole is often less than the sum of its parts. Despite eminence in their own fields, scholars in an interdisciplinary assemblage may add to entropy rather than information. The likelihood of recognizing that such has happened is greater for readers than for participants; the ceremonial rituals of a meeting in sumptuous surroundings create a rosy glow in which a set of disconnected arias is easily mistaken for an opera. After all, a symposium, according to the *Oxford Dictionary of English Etymology*, is a "drinking party" or a "convivial meeting for conversation". Not having shared the drinks, I am afraid I found myself progressively more discomfited by finding parallel play (in Piaget's sense) in *How Humans Adapt* when I had hoped for a game with rules and outcomes.

As academics, we comfort ourselves by referring to a community of scholars but perhaps it is not possible for a geneticist to find common language with an economist or an anthropologist with an historian except by so vulgarizing precise discourse that meaning is garbled. This conclusion is suggested by an interchange between Mary Midgley and Marx Wartofsky on the implications of sociobiology. Midgley argued that the doctrines of sociobiology do not amount to a revival of Social Darwinism despite her alarm about "the language, and the unconscious approach to the subject which it betrays. Sociobiologists often use words like 'selfish', 'altruistic', 'investment', 'strategy', and the like in such a way that, if those words had their everyday meaning, they would really be saying that each must always be only for himself and the devil must take the hindmost. They explain, however, from time to time, that they do not actually mean this" (pp. 524-5). Wartofsky countered that if "the sociobiologic argument yields a polyvalent, underdetermined basis of dispositions, among which the involved intelligence then chooses . . . the genetic program is, in effect, indifferent in determining cultural or moral choice. . . . If there are no independent grounds upon which the mapping from culture to gene or from gene to culture can take place, then the claim for the relevance of sociobiology is vacuous" (p. 537). Darwin himself despaired of finding a basis for morality in biology:

I own that I cannot see as plainly as others do,

and as I should wish to do, evidence of design and beneficence on all sides of us. There seems to me to be too much misery in the world. I cannot persuade myself that a beneficent and omnipotent God would have designedly created the Ichneumonids with the express intention of their feeding within the living bodies of caterpillars or that a cat should play with mice.

This symposium was planned on the assumption that knowledge of man's biological heritage and of the evolution of the species would shed light on how we ought to govern ourselves so as to traverse the present hazards to our survival: nuclear war, despoilation of the environment and population explosion. However, the wellsprings of unrest in the contemporary world, social inequality and the differential distribution of power, are not biologically determined, though they have profound biological consequences. Is science relevant at all, except in so far as it provides means for the ends which will be determined by the political process?

It is possible for biologists, as biologists, to look at the future of the entire species. In the words of Donald Ortner: "The future of civilized society is certainly threatened by (nuclear war). The threat, however, to the human species is very much less" (p. 143). Betty Meggers (p. 179) was more Olympian: "Natural selection is opportunistic and amoral. Pity and charity are alien concepts; 'ought' and 'should' are irrelevant verbs. Many more species have become extinct than are now alive We are unimportant in the history of our planet, which got along very well without us for several thousand million years. If we lose the flexibility to adapt, we too will become extinct. Other species will take our place, fill our niche, and carry on the evolutionary process — unless we, in passing, so alter the conditions for life that no existing organic forms can survive".

Meggers recognized the underlying political issues, which are hidden by a purely biological perspective: "But who are 'we'? The species, *Homo sapiens*? . . . Are 'we' the Chinese? The Brazilians? The Saudis? The Navahos? The !Kung Bushmen? If I judge correctly, 'we' are none of these; rather we are the intellectual products of Western civilization, represented by the participants in this symposium. In the context of the world today, much less of natural selection, the likelihood that 'we' will decide the future of the planet is vanishingly small" (p. 181). Her stark admonition had no discernible effect on subsequent discourse: it continued to flow in narrow disciplinary

channels, reminding one of Samuel Johnson's aphorism: "No man forgets his original trade: the rights of nations and of kings sink into questions of grammar, if grammarians discuss them". It was not until late in the meeting that Richard Barnet was able to deal with the interrelations between economic development, human rights, equity and democracy. Not surprisingly, he provided no neat answers. But he did identify the questions.

Evolutionary concepts dominated the discussion, with much of the debate centring on similarities and differences between biological and cultural evolution. In a fascinating chapter, James Neel traced out the reasons for loss in genetic diversity which followed upon the abandonment of the hunter-gatherer way of life with its differential fertility, inbreeding, and village fission along family lines. He noted the potential hazard to our species from genetic inertia, the preservation of deleterious genes (through improved medical care), and heightened vulnerability to new epidemic diseases. Yet, he doubted that genetic problems will or should attract much attention in the near future in the face of more immediate hazards to our survival. These challenges, he added, "will have to be met by minds essentially those of our primitive ancestors" (p. 87).

Neel recalled the problem which so perplexed A.R. (misprinted as A.E.) Wallace: "How could selection have established an organ with so much unused potential if the brain of 'savages' is equal in innate endowment to the brain of civilized Europeans?". Wallace, the co-discoverer of natural selection, was a hyperadaptationist; he believed that every feature of every organism was built for a direct adaptive purpose. He could not accept the notion that the human brain was constructed by natural selection before it was required by the demands of Western culture. Wallace fell back on divine intervention to explain its creation. Neel, just as Darwin did, dispelled Wallace's apparent dilemma by contending that: "The calculations that in primitive times entered into obtaining a second wife were no less demanding than aspects of the calculus, if less formalized, but with the additional flavor that the primitive man might lose his life if he calculated incorrectly" (p. 88). If that argument corrects for Wallace's ethnocentrism, it maintains the adaptationist assumption that the brain *must have been built* for the functions it exhibits because "I know of no parallel where nature has endowed us with a capacity far beyond our needs" (p. 306). The truth of this assertion is not at all self-evident. All change does not result from natural selection; some arises from random genetic drift, some from the constraints morphology and embryogenesis exert on the consequences of selection targeted elsewhere in the organism, some from still other processes. Yet these unselected

characteristics may later constitute adaptive advantage in the presence of changed environmental circumstances (Gould, S.J. and Lewontin, R.C. *Proc. R. Soc. Lond. B.* **205**, 581–598, 1978). Darwin, in the last edition of *The Origin of Species* (1872), protested against the misrepresentation that “I attribute the modification of species exclusively to natural selection”. He repeated what he had stated explicitly in the introduction to the first edition: “I am convinced that natural selection has been the main, *but not the exclusive means*, of modification”.

Despite the reservations expressed in this review, perhaps even because of them, reading *How Humans Adapt* proved to be stimulating. The book includes a number of excellent chapters: those by Jane and Chet Lancaster on parental investment, James Neel on human genetics, Betty Meggers on the control of human events, Nevin Scrimshaw on nutrition, Peter Laslett on demographic history, Richard Barnet on the future of democracy and James Gustafson on ethics; and incisive commentaries by Marx Wartofsky. In total, these contributions make up about one-quarter of the total pagination. At that, a one-to-three ratio of excellent to indifferent is probably above the mean for academic presentations on grand themes. If B.F. Skinner is correct that variable-ratio schedules of reinforcement are the most effective in maintaining behaviour, then it may be that it is the uneven and unpredictable interspersions of rewarding and unrewarding which keeps readers reading and reviewers reviewing. □

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Switched on to development

Julian Lewis

From Egg to Embryo: Determinative Events in Early Development.

By J.M.W. Slack.

Cambridge University Press: 1983.

Pp.241. £25, \$49.50.

EMBRYOLOGY, like many other areas of biology, is bedevilled by simplistic dichotomies. We need them, yet they trip us up. Our ways of thought, and the structure of human language, perpetually drive us towards descriptions based on discrete categories and simple yes/no questions. We can scarcely hope to understand the world in any other way. Thus, for almost a hundred years, it has been the tradition to ask whether an embryo is regulative or mosaic, whether its pattern is set up through intercellular interactions or through asymmetric cell divisions, whether a given cell is or is not determined for a certain fate, and so on. But the embryo is a complex system, changing gradually with time, fully describable only in terms of a large — and unknown — number of continuous variables; and the seemingly fundamental dichotomous questions have generated a great deal of confusion and conflict. The answers depend on the organism, the stage of development, the experimental criteria, the nuances of the definitions adopted, and the school of thought of the investigator. For the standard alternatives represent opposite extremes of a continuum, with real systems lying somewhere between; and a proper understanding of development requires a

theory of the underlying continuous dynamics. This is the implicit message of Jonathan Slack's book.

Its central concern is with the problem of “regional specification, . . . the process whereby cells in different regions of the embryo become switched onto different pathways of development”. It tackles this topic in the context of the early embryo, from the beginning of cleavage of the egg to the establishment of the basic body plan. These are the stages at which widely different species are most closely comparable and at which there seems to be the best hope of uncovering some universal principles. The focus throughout is on the mechanisms controlling the intrinsic characters of cells; the problems of morphogenetic cell movements are explicitly excluded by Slack from his discussion.

The book is written in three parts: first, an introduction presenting the traditional terminology of the subject, in the form of definitions with commentary; second, a review of the experimental evidence; third, a discussion of theories and models. The middle section is the longest, and makes the hardest reading. Amphibians, mammals, birds, insects and a variety of other invertebrates are presented systematically, with an account of the normal developmental anatomy preceding the evidence from experimental embryology for each. Much of this material is too compressed to be fit for beginners, but for those who already know something of the subject it is valuable as a concise comparative review, written in a lively, critical spirit. Passages of clear and illuminating exposition, as, for example, in the account of early insect development, make up for some tedium as one ploughs through such topics as the nomenclature of molluscan blastomeres.

The last part of the book is the most stimulating, and tackles effectively the anti-theoretical prejudices of myopic fact-collectors. (As Slack rightly says, “Those who have a nodding acquaintance with embryology often seem to regard [morphogenetic] gradients as some kind of mystical pseudoexplanation and a distraction from the serious business of biochemical analysis.”) Slack aims to show how the phenomena of regulation, determination, induction and so forth — the preoccupations of the previous chapters — can be interpreted in terms of the underlying chemical dynamics. He explains how symmetry-breaking instabilities in chemical reaction systems can spontaneously generate spatial patterns, converting an almost symmetrical egg into a highly asymmetrical animal. He discusses the genesis of discontinuities through thresholds in the responses of cells to a graded signal. He reviews reaction-diffusion models and considers mechanisms for the production of repetitive patterns such as the sequence of somites. The tone of the discussion is non-partisan as between competing theories,



Fever tree and swamp palm, by Jonathan Kingdon, taken from *Kilimanjaro: Animals in a Landscape* recently published by BBC Publications (pbk £5.95). The book is based on a recent television programme featuring Kingdon's work as an artist and zoologist, and coincides with an exhibition of his art at the Barbican, London, which runs until 4 December.

but thoughtful and often provocative. Many questions are posed for the reader to ponder, where other authors might peddle their own pet speculations.

The book as a whole contains much that one might quibble with (I would particularly quarrel with Slack's definition of a stem cell); it does not offer revolutionary new insights; it is not exhaustive — the

account of chemical reaction systems, for example, makes no mention of the possibility of chaotic behaviour. But it is entertainingly written; it gives a clear and refreshing perspective on a major problem; and, above all, it stimulates thought. □

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An alternative to life

Paul Calvert

An Introduction to Speciality Polymers.

Edited by Norio Ise and Iwao

Tabushi.

Cambridge University Press: 1983.

Pp.200. £22.50, \$42.50.

"SPECIALITY polymers" is a phrase which, like "pure research" changes its meaning with the context. In this case it means "polymers for non-mechanical applications". The field is enormous and this book is not a general survey but concentrates on synthetic polymer equivalents of active biological polymers including enzyme-like catalysts, photosynthetic systems, muscle-like polymers, polymers for selective transport, membrane formers and nucleic acid analogues. If you have been set the problem of creating an alternative form of life not based on proteins or nucleic acids, this is the book you need to begin.

It was originally published in Japanese in 1980, but has been updated and the membrane chapter added for the English edition. After a brief introduction there is a chapter by Makoto Okawara on reactive polymers. This essentially describes the tool-kit, surveying methods for producing polymer chains with a variety of attached reactive groups. There is nothing particularly revolutionary or all-embracing here but it is a useful review of what can be done.

Norio Ise reviews with great clarity polymers as catalysts, discussing the different types of interaction between the catalyst and the reactants. A second part to the same chapter by Iwao Tabushi covers attempts to produce polymers with an enzyme-like function. He flounders a bit in discussing what enzymic catalysis actually is when contrasted with other homogeneous catalysis. There are a variety of examples of low molecular weight catalysts which also work when attached to polymers but no sign as yet of any specifically "large molecule" effect.

Energy-converting polymers are dealt with by Shigeo Tazuke, who starts with muscle-like systems which convert chemical, thermal or light energy into mechanical energy by undergoing a conformational change. Interestingly the power densities and forces developed seem

to be similar to those of muscle though the only machines produced are in the category of intriguing toys. The implication would seem to be that biological design cannot compete in the energy-intensive artificial world. The rest of the chapter is on photochemical and photoelectric systems, touching briefly on many topics, but is not a coherent discussion. Data from an American paper on energy storage have crept through the double translation in the units of yen kg^{-1} . We need SI currency units.

Chapter Five by Manabu Senō gives a useful general survey of polymer membranes. He then goes on to talk about facilitated transport and active transport,

largely in biological systems, where the permeant is carried through the membrane by another molecule. A chapter on synthetic bilayer membranes follows, but does not really have much to do with polymers. The final chapter discusses information-transmitting polymers but does not get beyond a brief survey of nucleic acids and a few polymers with nucleosides attached to synthetic macromolecules. It really shows only that we have not got far.

This is apparently intended as a textbook but it is not really suitable for a polymer course; the approach is very biological and very variable. As a research book it is unsatisfactory, and misses out too much. The references are mostly general and many workers are mentioned with no reference at all. Part of this may be due to a desire to avoid referring to publications in Japanese. Despite these drawbacks there is a great deal here to stimulate thought. The patchiness of the treatment leaves one with a feeling of how much more could be done. □

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News of the growth business

J. Taylor-Papadimitriou

Growth and Maturation Factors, Vol.1.

Edited by Gordon Guroff.

Wiley: 1983. Pp.365. £51.75, \$72.50.

TWO of the major hazards facing authors writing a review on an expanding subject are, firstly, the possibility of new developments arising during the period from manuscript submission to publication and, secondly, the temptation to overemphasize work from the author's own laboratory. Although these hazards have not been completely avoided by the authors in *Growth and Maturation Factors Vol. 1*, the book does present a fairly comprehensive and balanced view of the properties and functions of some classes of growth factors. The most obvious omission is the recent data relating platelet-derived growth factor (PDGF) to the *sis* gene from simian sarcoma virus which has been well publicized. On the other hand, this development justifies the idea of producing a set of volumes to "present the unfolding information".

The material in the first volume tends to be organized around individual, or classes of growth factors. Where the growth factors themselves are well characterized as with epidermal growth factor (EGF), tumour promoters, nerve growth factor and PDGF, more space is devoted to recent data on the mechanism of their action. In these chapters, review of the literature and presentation of recent data is well balanced

and interesting. In each case, the area of active research is given emphasis so that the structure, processing and *in vivo* action of nerve growth factor complements the studies presented on membrane interactions of tumour promoters and internalization of EGF in culture systems. Clearly, the *in vitro* model systems using defined media described in the book are useful and productive for studying mechanisms of action of specific growth factors, but most studies have been done with fibroblasts and the growth factors acting on them (including the so-called transforming factors and PDGF).

Where the growth factors are members of a class, the emphasis is on data characterizing the factors and their relationship to each other. Growth factors are generally named after the function they promote but this can be as vague as multiplication stimulating activity (MSA) or colony stimulating factor (CSF), which may describe the activity of many factors. Further clarification is essential to unravel the complexity of growth factor interactions *in vivo*.

This first volume provides a good perspective on current knowledge and research into growth factors and their action, and should be useful to investigators and teachers requiring such a perspective. However, if the series is to be of interest to those actively involved in this field of research it may be important to concentrate on articles which integrate studies on different factors and to shorten the publication time. □

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Talk of echinoderms at Tampa Bay

David Nichols

Echinoderm Studies I.

Edited by Michel Jangoux and John M. Lawrence.

A.A. Balkema: 1983. Pp.200. Dfl. 70, £15.90.

BY ANY standards, the spiny-skinned echinoderms are successful marine organisms. Huge numbers of starfish are found in the rocky intertidal regions worldwide, sea-urchins are principal browsers in the shallow sublittoral reefs that border the world's oceans and, in deep water, sea-cucumbers can occur in such numbers that in places their biomass is said sometimes to exceed that of all other animals put together.

As most of them possess a fairly well-associated skeleton, they have been readily fossilized throughout Phanerozoic time and so the phyletic infrastructure of the whole group, with its five living classes and about 15 extinct ones, has received considerable attention, particularly given the plausible arguments put forward that echinoderms are the springboard for the evolutionary jump from invertebrates to chordates. Can such factors explain their fascination? Whether one regards them as unique in the animal world, enigmatic in their complexity, or simply convenient egg-factories for developmental and cell biologists, they have attracted increasing attention over the last 20 years.

Echinoderm specialists first met together at the 14th International Zoological Congress in Washington in 1963, and the resulting volume (Booolootian, R.A. (ed.), *Physiology of Echinodermata*, Wiley Interscience, 1966) was a monumental review of the group, system by system. At the most recent meeting at Tampa Bay, a series of plenary lectures was introduced, and *Echinoderm Studies I* contains expanded versions of these reviews. It is the first of an occasional series that will attempt to bring the Booolootian volume up-to-date, but narrowing the fields covered, and giving the authors more scope for speculation.

Three of the articles stress the convenience of echinoderms as subjects for the study of widely different phenomena in the marine environment. In the first place, they have unusually good regenerative powers. Early oyster-fishermen brought up starfish predators from the beds, tore them in half and flung them back into the sea; but it was not long before they realized that there was a good chance that both halves would regenerate, doubling the menace. In her article on phenotypic variation, Marcus reflects on this ability to produce genetically identical progeny: experimental work in this field often requires cloned

individuals and echinoderms represent potential subjects for such work.

Secondly, reflecting a younger field of study, the article by Craig on genomic variability in echinoids also concludes that this group, with its extensive fossil record indicating relationships and the ease by which radiolabelling can be performed, are particularly useful as subjects for mapping genome patterns.

Thirdly, an excellent review by Ebert points to the convenience of using echinoids in studying recruitment in marine communities, especially now that techniques exist for culturing the young stages and rearing them beyond hitherto impeditive metamorphosis.

The physiology and functional morphology of echinoderm respiratory systems have received relatively little attention and therefore Shick's wide-ranging review is most useful. He points particularly to the so-called 'internal problem': echinoderms have a large anaerobic component, perhaps accounting for the imbalance of certain energy equations in the massive internal tissues, especially when the gonads are ripe. Shick's treatment is an excellent example of the coherent blending of morphological and physiological aspects.

Echinoderms are not renowned for their learning ability, yet there have been reports of simple habituation which Valentinčič, in his contribution on innate and learned responses, suggests should be treated with caution. However, his own work on conditioning of a 'ready to escape' response in starfish is usefully covered.

One of the unique features of echinoderms is the array of semi-independent effector organs with which the outer body is endowed. Down among the spines are the defensive and cleansing pedicellariae, which have their own reflex activity, yet are linked to the 'central' nervous system for concerted action when required. Campbell gives a splendid review here, placing his own work in historical perspective, and showing the sophistication in miniature of these fascinating structures.

The echinoderms, distinct from all other animal groups in so many features but with a good fossil record, have naturally attracted the attention of palaeontologists who seek to understand their evolution. Sprinkle's article reviews some of the controversies that have arisen, including possibly the most contentious of all: were the fossil 'carpoids' really chordates? Sprinkle thinks this unlikely and that these highly unusual echinoderms represent convergence upon the 'flat-fish' design of asymmetrical adaptation to benthic living, presenting echinoderm workers with a remarkable group within an already unusual phylum, whose puzzles and challenges are well reviewed in this book. □

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Mathematics for physics

Michael Atiyah

Topology and Geometry for Physicists.

By Charles Nash and Siddhartha Sen. *Academic Press: 1983. Pp. 310. £25, \$39.50.*

GEOMETRY and physics have a long and entangled history. Euclidean geometry, with the help of Descartes, provided in due course the framework for the Newtonian Universe. Later, in the nineteenth century, the discovery of non-Euclidean geometries liberated mathematicians and led to a much wider range of geometrical thinking. One aspect of this was Riemann's differential geometry which eventually provided the mathematical foundations of Einstein's General Theory of Gravitation. A different but related strand was the development of the geometry of 'fibre bundles' by Elie Cartan and his followers. Together with the associated growth of topology (which might be described as 'qualitative geometry') this has been one of the most vigorous and successful branches of twentieth-century mathematics.

Quite remarkably all this modern geometry and topology, although apparently very abstract, has suddenly come into prominence, as it provides the mathematical setting for the latest and most promising theory of elementary particle physics — the so-called gauge theories. As a result physicists have found it necessary to come to terms with modern geometrical ideas, a task made more difficult by the different technical jargon employed by mathematicians and physicists.

Mathematical books are notoriously difficult to read. The requirements of formal proof lead to a ponderous and intimidating style, and there are few books that could be recommended to the enquiring physicist. It is to remedy this situation that Nash and Sen have written their book. It is intended for the physicist who would like to learn the rudiments of modern geometry and topology. As a result there is much more in the way of motivation and illustrative examples than is customary. Moreover the final chapters deal specifically with physical applications in both solid state and gauge theories.

Although the style is less formal than in standard treatments, the coverage is quite extensive and thorough, and the book could usefully be read by mathematics students. Altogether the authors have achieved their purpose and provided a work which should prove extremely useful in bridging the gap between geometry and physics. □

Sir Michael Atiyah is at the Mathematical Institute, University of Oxford.

American Society for Cell Biology

To mark the American Society for Cell Biology's meeting in San Antonio, Texas (29 November to 3 December) this selection features items used in cell biology

Biologicals

● A monoclonal antibody to the leucocyte common antigen is now available from Dako that can stain formalin-fixed paraffin-embedded material. It is for use in routine histopathological specimen processing. This anti-leucocyte may be used to detect white cells in tissue sections. It is suitable for identifying tumours of lymphoid origin and for distinguishing them from non-haematopoietic neoplasms such as carcinomas, sarcomas, and melanomas.

Circle No. 100 on Reader Service Card.

● A new kit for the measurement of adrenal corticotrophic hormone (ACTH) is now available from Walker Laboratories. The kit has a standard range of 25 to 1,000 pg ml⁻¹, with a detection limit of approximately 10 pg ml⁻¹. Two control plasmas are included in each kit and individual components can be ordered separately.

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● A new radioimmunoassay (RIA) kit has been produced by Walker Laboratories for use in measuring myelin basic protein in cerebrospinal fluid samples. The kit contains reagents for 50 tests and is complemented by the creatine kinase B (CK-B) assay. Individual components from both CK-B and myelin BP are available.

Circle No. 102 on Reader Service Card.

● Metachem Diagnostics has introduced the new human diagnostic GPO-PAP (glycerol 3-phosphate oxidase peroxidase anti-peroxidase) triglycerides test. This new enzymatic colourimetric test is linear to 11.4 mmol per litre, with no interference from haemoglobin or bilirubin until gross levels are reached. Once reconstituted, the reagent is stable for 14 days if stored between 2 and 8°C, or for 3 days if stored at room temperature. The reagent is intended to be used on an automatic analyser, as the incubation time is short and there is no need to use a sample blank.

Circle No. 103 on Reader Service Card.

● Worthington Diagnostic Systems is now marketing a purified cellulase product that isolates viable protoplasts from most plant tissues. It can be used for the isolation and culture of plants such as soybean, maize and red beet which can be difficult to culture otherwise. Two formulations of the product can be supplied. The first is a tan lyophilized powder from which most salts and low molecular weight contaminants have been removed. It has an Avicel activity of 25 to 45 units per mg dry weight. The second is a near-white version which is salt-free, having been purified chromatographically. This second version has an activity of 45 to 60 units per mg dry weight. Both formulations will remain stable if stored at 4°C.

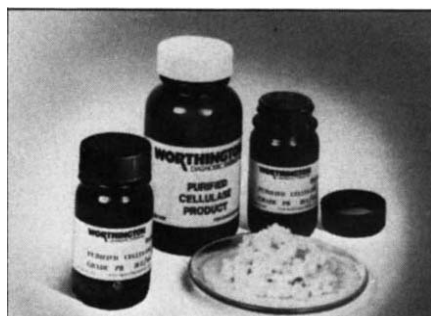
Circle No. 104 on Reader Service Card.

● Biomedica has made an addition to its HistoScan series of immunohistochemical staining kits. The new kit is designed to recognise the presence of keratin in paraffin-embedded as well as in frozen tissue sections. This new keratin immunoperoxidase kit follows the principles of avidin-biotin affinity cytochemistry, which means that the incubation steps are very short. This kit contains the basic reagents needed to stain 150 tissue sections up to the mounting stage. The reagents are supplied, ready-to-use, in colour-coded dropper bottles. Stained sections can be completed in 40 minutes.

Circle No. 105 on Reader Service Card.

● Cellular Products is now producing a natural human B-cell growth factor (BCGF). The product is claimed to be free of inducing agent (PHA-P), immune interferon and T-cell growth factor. It will support both short- and long-term proliferation of human B-cells in culture.

Circle No. 106 on Reader Service Card.



Worthington's cellulase product

● Natural human T-cell growth factor is now available from Cellular Products. It is obtained from a pool of at least 100 blood donors and each lot is tested for long-term growth. The growth factor is available either in phytohaemagglutinin-induced and delectinated form or as terephthalic acid-induced, delectinated, serum-free and protein-free.

Circle No. 107 on Reader Service Card.

● The radioisotope division of the ICN Life Sciences group has produced all four P³²-labelled deoxyribonucleotides at two specific activity ranges. Both the 600 Ci mmol⁻¹ and the 2,000–3,000 Ci mmol⁻¹ range are purified by preparative HPLC. The solutions are supplied at an initial concentration of 10 mCi per ml. New batches of the nucleotides are produced each week.

Circle No. 108 on Reader Service Card.

● Gibco has produced a kit for preparing serum-free media. This Select-a-Factor kit contains 1 ml sample sizes of the more commonly-required factors and technical information on how to grow cells in a serum-free environment. The technical information describes four experiments that explain the preparation of serum-free media. The kit includes 1 litre of powdered medium (Dulbecco's minimum essential medium: nutrient mixture F-12 with 15-mM HEPES), trypsin inhibitor and 14 ready-to-use factors (EGF, FGF, fibronectin, poly-D-lysine, vitamins C and E, insulin, transferrin, hydrocortisone, progesterone, selenium, putrescine, trace element mix, and T₃). This range is provided in the initial kit so that the correct mix can be determined, but the factors are also available singly for making up subsequent media.

Circle No. 109 on Reader Service Card.



The Select-a-Factor kit from Gibco

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● The new protease substrate gel tablets from Bio-Rad provide a method for preparing agar diffusion plates for use in detecting protease activity. The tablets can be used to detect contaminating proteases in purified protein or nucleic acid preparations, as well as to look for plasmin, the serum enzymatic contaminant.

Circle No. 110 on Reader Service Card.

● Five growth factors are now available from Genzyme. These are human interleukin 1, human interleukin 2, colony stimulating factor (mouse), colony stimulating factor (human) and mouse nerve growth factor. Collagen II (chicken) may also be supplied.

Circle No. 111 on Reader Service Card.

● It is now possible to quantify T and B cells in a single tube assay with Bio-Rad's Quantigen T & B cell assay. Sample preparation takes 2½ hours, using stable matched reagents. A light microscope is used for counting. The Bio-Rad T & B cell assay uses antibodies coupled to microbeads as cell labelling agents. This eliminates the multiple washes required by second antibody techniques. Single tube assay allows the number of 'double-marker' cells to be counted.

Circle No. 112 on Reader Service Card.

● NMS Pharmaceuticals has introduced a new human chorionic gonadotropin (HCG) assay. The new assay features a 75-minute quantitative procedure and a 20-minute screen, neither of which use a second antibody incubation. Sensitivity at 90% binding is 2 mIU ml⁻¹. The screen assay uses a 10 or 25 mIU ml⁻¹ cutoff and one single incubation period. Initially, kits are shipped with a total count of greater than 100,000 c.p.m. Stability studies have shown total counts to be greater than 60,000 c.p.m. on the expiry day.

Circle No. 113 on Reader Service Card.

● BioGenex Laboratories has introduced two new immunohistology kits. The first, called HistoGen adenocarcinoma, is designed for the positive identification of adenocarcinomas of the lung, colon, stomach and breast. The second, called HistoGen lung carcinoma, is designed for the positive identification of carcinomas of the lung and adenocarcinomas of the colon, stomach, breast and pancreas. Both kits use monoclonal antibodies and the indirect immunoperoxidase staining technique, thus providing a means of detecting antigens.

Circle No. 114 on Reader Service Card.

● American Bio-Nuclear now offers an oligonucleotide synthesis service. The new service can provide oligonucleotides of up to 35 bases to laboratories that are not equipped for DNA synthesis. Catalogues and samples can be provided on request.

Circle No. 115 on Reader Service Card.

General laboratory equipment

● A new two-position screw cap has been produced by Nunc. It can be vented, which prevents the flasks bowing. This can sometimes occur when they are placed in the incubator. The new caps have uniform and free air passage that counteracts positive pressure.

Circle No. 116 on Reader Service Card.

● A small, glass volumetric flask with an open screw cap has recently been introduced by Pierce. The sample is retrieved through a Teflon-faced disc thereby avoiding exposure of the sample. The flasks are made of borosilicate glass and are available in 2, 3, 5 and 10 ml sizes.

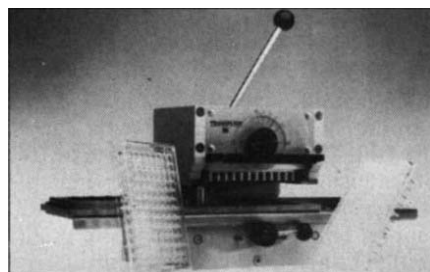
Circle No. 117 on Reader Service Card.

● The model 260 from World Precision Instruments is a dual-channel constant-current source for the electro-injection of drugs, dyes and histological fixatives at the cellular level. It is powered by 9-V transistor batteries and can generate a constant current in two ranges: 0 to 100 nA and 0 to 1 µA.

Circle No. 118 on Reader Service Card.

● Brinkmann Instruments has introduced a new centrifugal grinding mill, the ZM-1. This can break down almost any dry sample with a hardness of less than 6 mohs in volumes from 3 to 500 ml. This can be extended to 5 litres with an optional cyclone collection chamber. The mill has interchangeable rotors and sieves that can reduce materials to a specific particle size down to 40 µm. The grinding mill has a dual-speed motor (10,000 and 20,000 r.p.m.), a 60-minute timer, a speed-selector switch and an amp meter. Applications include investigating seeds for insecticides.

Circle No. 119 on Reader Service Card.



The Transplate⁹⁶ from Costar



Brinkmann's grinding mill

● Whatman has introduced an additional colour-bonded pH indicator covering the range pH 4.5 – 10. Each plastic strip of the indicator has three separate paper test fields fixed to one end. The indicator reagents are chemically bonded to the paper and consequently are not leached into the solution being tested. Each box of 100 strips contains a colour chart graduated in 0.5 of a pH unit which allows more accurate readings to be taken around the point of neutrality.

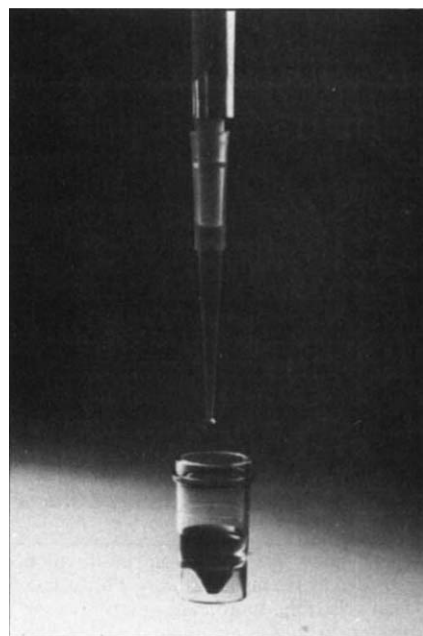
Circle No. 120 on Reader Service Card.

● Transplate⁹⁶ is a new pipettor from Costar that uses a disposable sterile cartridge. This pipettor removes liquid from or dispenses liquid into all 96 wells of a standard plate simultaneously. The unit is continuously adjustable for any volume from 25 to 200 µl and is reproducible within ± 2%. Options for the system include an incremental dispensing capability and disposable reagent reservoirs.

Circle No. 121 on Reader Service Card.

● Elkay's range of pipette tips has been extended to include cadmium-free tips, designed for use with Pipetman liquid transfer pipettors. The new Elkay tips fit Pipetman model P-20, P-100 and P-200 up to 200 µl. To correspond to the pipettor manufacturers specification, the tips are yellow and have a capacity of 1–200 µl. They are packaged in Elkay's Conve-Rack pack which consists of five autoclavable trays holding 200 tips, each with an integral tray and pack covers. Using these trays, the tip can be attached directly to the pipettor without handling, thus ensuring the tip is chemically clean before drawing the sample.

Circle No. 122 on Reader Service Card.



Elkay's cadmium-free pipette tips



Erlenmeyer flasks from Kontes

● The Turner model 20 photometer from Techmation monitors biocide treatment by luminescence determination of microbial levels in industrial aqueous or gelled fluids. Stable reagent kits are provided for field and laboratory use. The instrument is built for use in the field and can be operated from a laboratory a.c. power source or from a d.c. inverter.

Circle No. 123 on Reader Service Card.

● Kontes has added a new screw-cap Erlenmeyer flask to its line of Microflex products. The new cap is available in 10 and 25 ml sizes, with graduations and a writing area marked. A Teflon-lined septum provides an inert seal and a hole in the cap allows access with a syringe.

Circle No. 124 on Reader Service Card.

● Labconco has introduced a new class II biohazard safety cabinet, called the Purifier. It is designed for work with low to moderate toxic agents and protects the operator from hazardous work materials. It also provides a contamination-free environment for the work materials. Room air drawn across the face of the cabinet is immediately pulled downward into the cabinet without reaching the work area and is then filtered. The system filters out particles 0.3 μ m in size.

Circle No. 125 on Reader Service Card.

● A new range of temperature-controlled baths and circulators has been introduced by Haake. The removable control heads can be turned in any direction. The new Haake circulators incorporate a digital thermometer, which operates independently of the digital temperature control. The instruments have two temperature sensors, one for temperature control and one for temperature measurements. The circulators cover a temperature range of -90 to 300°C. They can give a control accuracy of $\pm 0.01^\circ\text{C}$.

Circle No. 126 on Reader Service Card.



Brinkmann's pestle and mortar mill

● A new version of the low temperature incubator is now available from Astell Hearson. The incubator control panel of the mark IV has been re-styled to accommodate a defrost warning light and new temperature setting dials. The drum thermometer has been replaced by a digital LED temperature display. The temperature safety thermostats are now calibrated in degrees centigrade.

Circle No. 127 on Reader Service Card.

● A new microtitration plate washer, the SLT 220, has been introduced by Kontron. The SLT 220 is a 96-channel washer capable of removing all free components in microtitration plates using two different washing solutions and up to three wash cycles. Wash cycles can be programmed by the user. Each cavity of the microtitration plate has its own injection system essentially eliminating cross contamination. The washing solution is injected into all 96 cavities simultaneously and fluid is removed continuously.

Circle No. 128 on Reader Service Card.

● A mechanized pestle and mortar mill that can be used for grinding biological materials has been introduced by Brinkmann Instruments. Most materials of particles up to 8 mm in size can be ground to diameters of from 1 to 20 μ m in either wet or dry processes. Total sample capacity can be up to 23 ml.

Circle No. 129 on Reader Service Card.

● Applikon has recently issued a new line of small fermentors and speed controllers under the name ASA Dependable Instruments. The fermentors have an accessible top-plate with five apertures for temperature, anti-foam, pH/redox, CO₂, and turbidity sensors. They have five vessels for cell cultures and continuous cultures: either flat, round or double walled.

Circle No. 130 on Reader Service Card.



Cambridge Technology's Platewasher

● A new system for washing microtitration plates has been introduced by Cambridge Technology. The new model 260 Platewasher is a semi-automated system for washing the wells of microtitration plates before they are read. The flow to each of the 24 wash needles shuts off individually and simultaneously. The Platewasher is made primarily of aluminium and stainless steel parts and will accommodate all common wash fluids. A work station for all standard 96-well microtitration plates is provided, as well as adjustable stops for use in wells of various depths. The wash head is magnetically stored in its own chamber.

Circle No. 131 on Reader Service Card.

● Astra Scientific International has introduced a new, microprocessor-controlled amino acid analyser. The instrument can run protein hydrolyzates in 55 minutes and physiological fluids in 120 minutes from injection to injection. Up to eight programs can be stored, then run in any combination or sequence. All important program parameters are monitored and displayed digitally at all times. The system includes a high performance cation exchange column.

Circle No. 132 on Reader Service Card.

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Circle No. 62 on Reader Service Card.

Specialized laboratory equipment

● The Hamilton Prep-Set is a new sample preparation system. It comprises 50-ml polypropylene tubes with screw caps, a sample rack, a milling sphere of either stainless steel or tungsten carbide and a push-through filter cylinder which accepts various filter discs. Prep-Set allows solid samples to be milled, extracted and filtered in individual tubes and thus avoids possible cross-contamination. The sample rack holds ten tubes and, with the filter plunger press, allows all samples to be filtered simultaneously. Prep-Set can also be used for dissolution, liquid-liquid extraction, sedimentation, centrifugation or incubation. *Circle No. 133 on Reader Service Card.*

● Balzers Union has introduced a new range of high vacuum freeze etching systems, designated BAF 500K. The BAF 500K systems feature an automatic cryopumping arrangement; a vacuum of 5×10^{-10} mbar and a refrigerator cold table, with temperature adjustment from 20 to -265°C without liquid helium. The working vacuum is reached 10 minutes after the specimen has been introduced. *Circle No. 134 on Reader Service Card.*

● RIA (UK) are introducing a three-level gas chromatography, mass spectroscopy (GCMS) steroid control serum to the UK. This serum, Gamma-B tri-GCMS, gives an absolute rather than consensus value for all steroid assays. It has been developed for laboratories using either tritium, extraction steroid assays or ^{125}I direct methods. Sets of Gamma-B tri-GCMS include six vials, each containing 2 ml of lyophilized human serum. The serum has a shelf life of 2 years. *Circle No. 135 on Reader Service Card.*

● Malthus Instruments has produced a microbiological growth analyser, called the 112 Clinical System. It provides an automated method for detecting the presence of microorganisms by monitoring the change in conductivity caused by their growth. Data are collected, stored and processed in the system's minicomputer and are displayed on a VDU or chart recorder. The system uses autoclavable sample cells of 2, 10 and 100 ml capacity. It has a modular design which means that it can be expanded to include up to 364 samples. *Circle No. 136 on Reader Service Card.*

● An instrument is being marketed by Dianorm that will prepare homogenous unilamellar liposomes on a microscopic scale. The instrument, called Mini-Lipoprep, can be supplied with one of two different cell sizes with nominal volumes of either 0.2 or 1.0 ml. Two other instruments are also being produced that will prepare larger liposomes. These are the Lipoprep and the Lipoprep-CLS. *Circle No. 137 on Reader Service Card.*

● Shandon Southern Products has introduced a new range of consumables for histology and cytology. The new range includes: stabilized haematoxylin and Harris haematoxylin stains, blueing reagent, histoplast paraffin embedding wax, histosol clearing agent, a safer substitute for xylene and a range of microtome knives. *Circle No. 138 on Reader Service Card.*

● A new high intensity ultrasonic cell disrupter is now available from Sonics & Materials that is capable of energizing two converters simultaneously. Several accessories designed for ultrasonic cell disruption, homogenization and emulsification can also be supplied. *Circle No. 139 on Reader Service Card.*

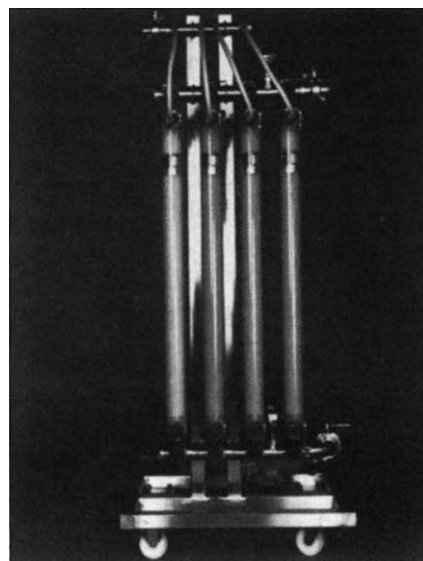
Filters

● A new 4-mm Millex-HV filter unit for small volume HPLC sample clarification is now available from Millipore. It is designed to improve recovery of samples under 1 ml, providing a hold-up volume of less than 10 μl after air purge. Like the standard 25-mm Millex-HV unit, the new Millex-HV4 incorporates the 0.45- μm hydrophilic Durapore membrane, which can filter most organic or aqueous samples. The unit will retain all particles above the rated pore size. *Circle No. 140 on Reader Service Card.*

● Millipore's new Minitan ultrafiltration system is a tangential-flow device for concentrating and desalting small volumes (200–3,000 ml) of macromolecules, viruses, or mammalian cells. The Minitan system eliminates the transfer of biological materials from one vessel to another and will process volumes of 200–3,000 ml down to 25–30 ml. The speed at which the Minitan system concentrates and desalts proteins such as antibodies, serum proteins, lymphokines and interferons prevents the proteins becoming denatured. *Circle No. 141 on Reader Service Card.*

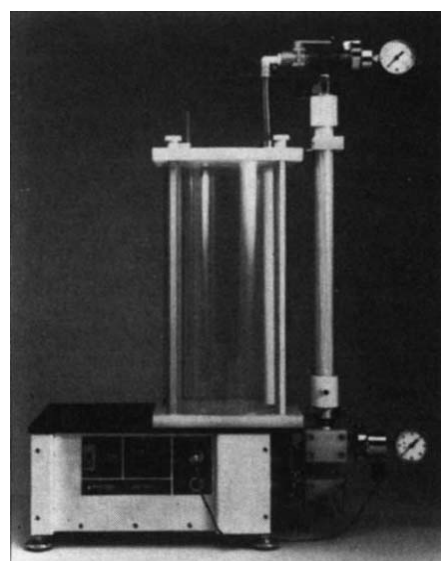
● Romicon has introduced a new ultrafiltration system for both pilot plant and small scale production operation. The HF-4 is designed to accept either Romicon's long (26.5 ft^2), short (15 ft^2) or short-short (7.5 ft^2) hollow fibre membrane cartridges. *Circle No. 142 on Reader Service Card.*

● A new filtration system that is capable of simultaneously processing 96 individual samples is now available from Millipore. The Millititer filtration system consists of a membrane filter sealed to the bottom of a standard 96-well plate and a vacuum filtration holder. When used as a filtration device, the Millititer plate will retain all particles or cells larger than its rated pore size. Using the vacuum filtration holder, the filtrate can be directed into a standard 96-well plate or disposable collection tray. The membrane can also serve as a substrate for the immobilization of proteins and nucleic acids. *Circle No. 143 on Reader Service Card.*



The HF-4 from Romicon

● The new DC10L hollow fibre ultrafiltration system from Amicon has been designed to allow the processing of between 5 and 50 litres of cell suspensions or of macromolecular solutions. The system will process 20 litres of bacteria, yeast or cells. It includes a reservoir, a stainless steel positive displacement gear pump and a pre-filter. An optional liquid level control unit can be fitted for unattended operation. The basic system uses a single Amicon 5 ft^2 (0.45 m^2) or 10 ft^2 (0.9 m^2) hollow fibre cartridge, although this can be expanded to allow two cartridges to be used. *Circle No. 144 on Reader Service Card.*



Amicon's DC10L ultrafiltration system